"Sapienza" University of Rome PhD school in Neuroscience

PhD program in Behavioral Neuroscience

Curriculum in Psychobiology and Psychopharmacology

Role of dorsal and ventral hippocampus in working memory capacity

Academic Year 2017/2018

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Introduction

Memory and the different memory systems

Memory is a fundamental process for human beings, since what we remember determines largely who we are. Memory is defined as the current knowledge about something that was presented previously (Robertson 2002).

Neuroscientists and experimental psychologists distinguish several types of memory (Fig.1), each of which is served by different combinations of brain regions (Wood, Dudchenko et al. 1999).

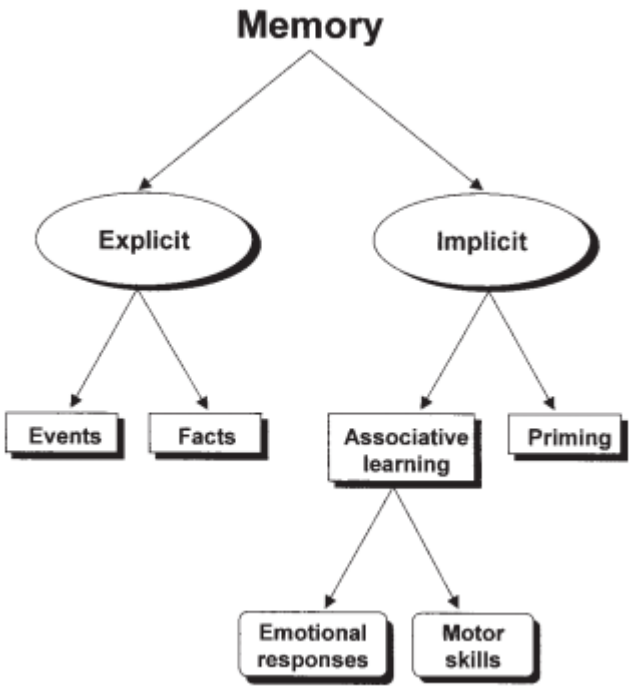


Fig. 1. *Memory can be classified into two major types and several subtypes. Explicit memories are those events and facts that can be consciously recalled. Implicit memories are skills, habits, and information that are acquired and retrieved unconsciously (Robertson 2002).*

Two general kinds of memory are described: 1) *explicit* memory which is conscious recollection of one's own previous experiences and, 2) *implicit* memory which is past experiences that influence current behaviour but are not consciously recalled. Explicit memory, referred to as simply "memory" in ordinary language, can be further subdivided into events that are personally experienced (that is

episodes of our life) and memories containing factual information (that is our general knowledge). The first are known as *episodic memory*, the second are known as *semantic memory*.

Implicit memories include motor skills and emotional associations with particular stimuli or events, which form our likes and dislikes. Implicit memories also include *priming* which is the ability to identify an item as a result of previous exposure to it, even if you are unconscious of the previous exposure.

Different regions of the brain participate in the encoding, storage, and retrieval of particular experiences, events, facts and skills. Some of the first insights into where and how the brain processes memory came from the study of brain-injured amnesic patients (Scoville and Milner 1957). In 1957, Scoville and Miller published a landmark case study of a twenty-seven-year-old man (Hanry Molaison, H.M.) with a history of epilepsy, who underwent a neurosurgical procedure to bilaterally remove the medial temporal lobes (MTL) including the hippocampus (HP) and the amygdala that lie deep within the lobe. The surgery successfully eliminated the seizures, but immediately after the surgery, H.M. was severely amnesic of events leading up to his operation and he had a profound inability to learn and retain any new memories of facts and events. Extensive psychological testing revealed that H.M.'s personality, perception, and intelligence did not change, nor did he have problems with learning new motor skills. However, H.M. was completely unable to form any explicit memories after surgery. The findings from H.M. established three fundamental principles that continue to guide experimental work. First, memory is a distinct cerebral function, separable from other cognitive abilities. Second, because H.M. was able to retaining information for a short time, the MTL is not needed for immediate memory. Third, the structures damaged in H.M. are not the ultimate repository of memory, because he retained his remote childhood memories (Squire 2009). It subsequently became clear that only one kind of memory, *declarative memory* or explicit memory, is impaired in H.M. and other similar patients (Cohen and Squire 1980). Thus, memory is not a unitary faculty of the mind but is composed of multiple systems (Broadbent, Squire et al. 2004). Studies in the monkeys identified the anatomical components of the MTL system as structures that support declarative memory (Parkinson, Murray et al. 1988). The monkeys were the first animal models of human impairment.

Similarly, the brain systems that support various kinds of *non-declarative* memory or implicit memory came under study. The best-understood example of non-declarative memory in vertebrates is classical conditioning of the eyeblink response, specifically delay eyeblink conditioning. The essential memory trace for the conditioned eyeblink response is formed and stored in the cerebellar interpositus nucleus (Mccormick, Lavond et al. 1981). Shortly after the cerebellum was linked to

conditioning, the striatum was proposed to be important for habit learning (Mishkin and Petrie 1984). Subsequently, a dissociation between brain regions regulating declarative memory and habit memory was demonstrated in rats with fornix or caudate lesions who were given two tasks that appeared to assess declarative memory and habit memory, respectively (Packard, Hirsh et al. 1989). Only rats with fornix lesions were impaired in the first task, and only rats with caudate lesions were impaired in the second task. A similar contrast between declarative memory and habit memory was described for memory-impaired patients with HP lesions and patients with nigrostriatal damage caused by Parkinson's disease (Knowlton, Mangels et al. 1996). Finally, the evaluation of positive or negative valence of a stimulus (associative learning) is a form of non-declarative memory strictly dependent on the amygdala.

Time-dependent multi-stages of memory

Explicit memories are also classified according to time. This temporary subdivision of memory was proposed by Aktinson and Shiffrin (Aktinson and Shiffrin, 1968) in the Multi-Store model of memory. He postulated that there are three different registers of memory: ***sensory memory, short-term memory and long-term memory.***

Information encoded from our senses is initially stored in sensory memory for few seconds, which is long enough for us to decide which parts of it are important enough to transfer to short-term memory. Information is stored in short-term memory the time we repeat it, a phenomenon known as *rehearsal*. If we rehearse and use information, it can be kept in ***working memory***. Depending on the extent of rehearsal or use, memory is either discarded or planted in the long-term memory (Fig. 2).

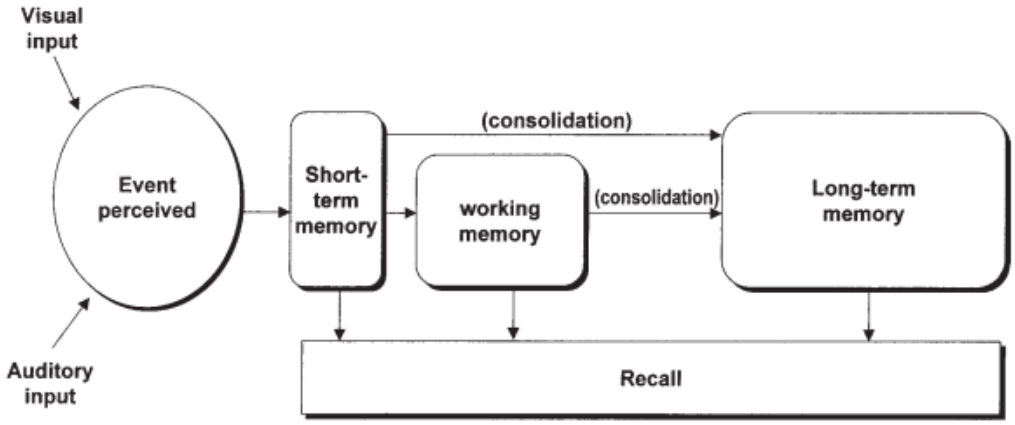


Fig. 2. A model of how the brain stores explicit information (Robertson 2002).

Long-term memories are for recalling specific events and facts, recognition of people and locations, and particular skills, which can be retained for a long period, especially if revisited periodically. Retrieval is commonly known as remembering or recall. A subset of long-term memory is ***remote memory*** or ***long lasting memory*** that include deeply embedded knowledge as the language which are often the last memories to be lost in conditions such as Alzheimer's disease.

An important question has been whether short-term memory, working memory, and long-term memory are simply different phases of long-term memory or are separate and parallel phenomena. Hebb and Gerard (1949) proposed dual-trace theories of memory, suggesting that the stabilization of the neural activity underlying short-term memory produces long-term memory (Gerard 1949, Hebb 1949). This supported the idea that short-term memory is only a phase of long-term memory. On the contrary, the finding that protein synthesis inhibitors did not prevent the learning of tasks but disrupted memory of the training supports the view that there are (at least) two stages of memory and indicates that protein synthesis is required only for consolidation of long-term memory (Fig. 3).

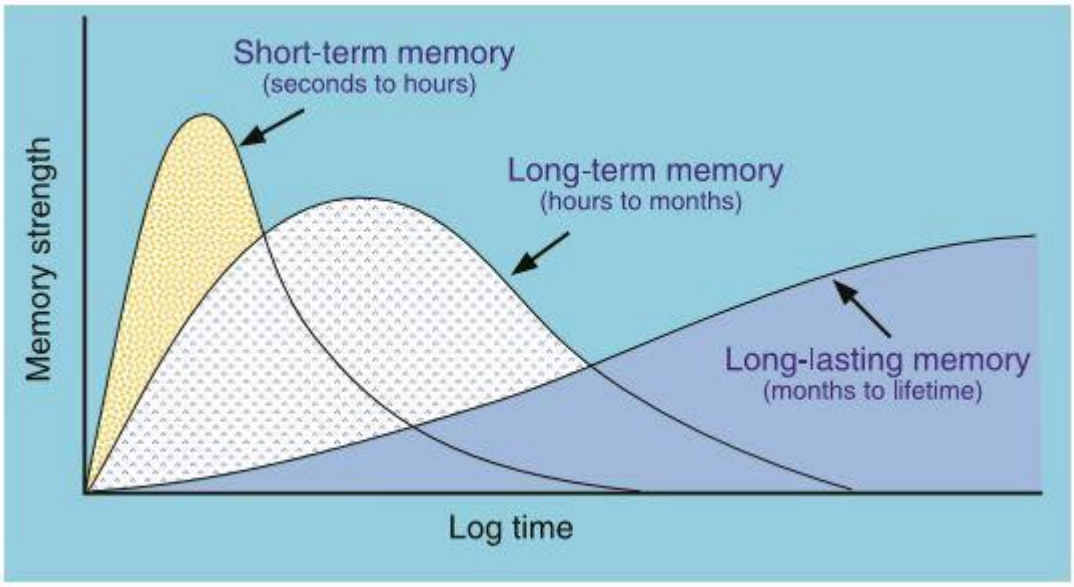


Fig. 3. Memory consolidation phases. *Short-term and different stages of long-term memory are not sequentially linked but they are independent processes acting in parallel (Mc Gaugh 2000).*

According to the theory that the time categories of memory are not phenomena sequentially occurring but they are independent and parallel, the case of H.M. patient demonstrated that the surgical bilateral destruction of the temporal lobes did not affect all the categories of memory but only those brain structures that transfer short-term memories into long-term memory.

The transfer of short term memories into long-term memories was defined **memory consolidation.** The standard idea is that memory consolidation begins when information, initially registered in the neocortex, is bound into a memory trace by the HP and related structures in the MTL (entorhinal, perirhinal, and parahippocampal cortices) and diencephalon (Squire LR and Zola-Morgan S, 1991). This initial binding of information into a memory trace involves a *short-term consolidation* process, or cohesion, that is believed to be completed within seconds or, at most, tens of minutes. A process of *long-term consolidation* then begins. At first, the HP and related structures are necessary for the storage and recovery of the memory trace, but their contribution diminishes as consolidation proceeds, until the neo-cortex alone is capable of sustaining the permanent memory trace.

Finally, **retrieval**, commonly known as recall, is a process that occurs when brain activity comes to resemble the brain state that was present during original learning. During retrieval of a memory, various brain areas are simultaneously activated, a process that occurs within milliseconds, which results in a unified memory in our consciousness. The HP and the entorhinal cortex also participate to the process of *retrieval* of a memory (Polyn, Natu et al. 2005).

Working memory (WM) and its neural basis

Different brain regions regulate short-term memory and working memory. The distinction between short-term memory and working memory was an important point of memory classification. While short-term memory may be used to briefly hold in mind some information, working memory (WM) is responsible for the short-term storage and online manipulation of information necessary for higher cognitive functions, such as language, planning, and problem solving. It is a term that was used by Miller et al. (1960) to refer to memory as it is used to plan and carry out behaviour. The term became much more dominant in the field after Baddeley and Hitch (1974) demonstrated that a single module could not account for all kinds of temporary memory. Their thinking led to an influential model (Baddeley, Logie et al. 1986) in which verbal-phonological and visual-spatial representations were held separately, and were managed and manipulated with the help of attention-related processes, termed the *central executive*. The verbal-phonological representations are held in the *phonological loop* while visual-spatial representations are held in the *visuo spatial sketch pad* (Fig.4).

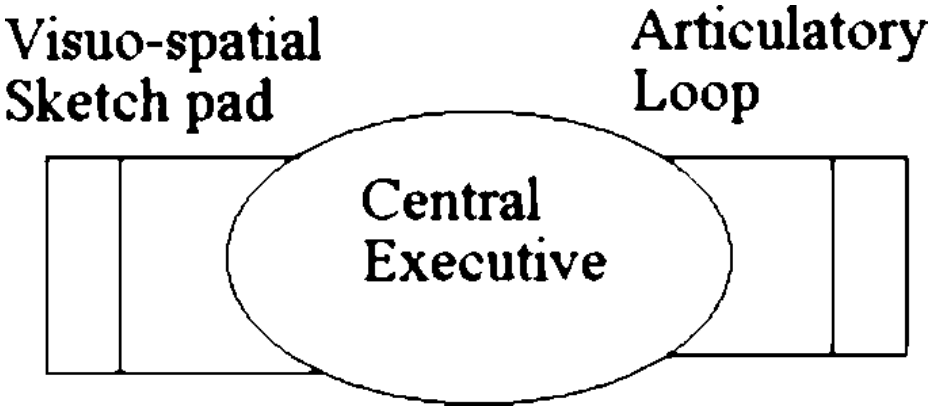


Fig. 4. Baddeley and Hitch's 1974 working memory model

By 1986 Baddeley added another component, the *episodic buffer* that seemed necessary to explain short-term memory of features that did not match the other stores (particularly semantic information in memory) and to explain cross-domain associations in WM, such as the retention of links between names and faces. WM is usually divided into two types of processes: "active maintenance" which is keeping information available, and "executive control" which governs the encoding and retrieval of information in working memory (Fuster 2000).

The main distinction between short-term memory and WM was the major demand on the attention required by WM tasks. In fact, the newer WM tasks correlate with intelligence and aptitude measures so much more highly than do simple, traditional, short-term memory tasks such as serial recall (Cowan 2008). Kane et al. (Kane, Bleckley et al. 2001) found that individuals scoring high on storage and processing tests of WM are less vulnerable to distraction. So, the new definition allows the simple statement that WM correlates highly with aptitudes, whereas short- term memory does not specifically correlate with aptitudes.

Distinct regions within the prefrontal lobe appear to handle the two types of WM, with the executive control processes being handled in anterior and ventral parts of the lobe, and the content-specific information (such as verbal versus visuospatial) sub-served by the cortex in the more dorsal and posterior regions.

First studies in animals associated WM with the prefrontal cortex (PFC) (Pribram, Mishkin et al. 1952) and this link was later confirmed by electrophysiological recordings that revealed neural correlates of WM in the PFC of monkeys (Fuster and Alexander 1971, Kubota and Niki 1971). The cellular basis of WM has been investigated extensively with electrophysiological recordings in macaques while they remember the spatial location or identity of a stimulus over a delay period lasting for a few seconds (typically 1–5 s). Such investigations reveal that single neurons in the PFC exhibit

discharges that persist even after the physical stimuli that elicited them are no longer present. Persistent activity during the delay period of WM tasks is thought to be generated by reverberating discharges in a network of interconnected neurons in the PFC, and between the PFC and other brain areas, such as the posterior parietal (Fig. 5A) and inferior temporal cortex, and subcortical structures, including the basal ganglia and the mediodorsal nucleus of the thalamus. Lesions of the prelimbic region, a part of the cortex that is suggested to be a homologue of the primate PFC, impair performance on WM tasks in rodents (Baddeley and Hitch 1974).

Performance of WM tasks is associated with activity in a wide range of areas across the human brain, depending on the type of stimulus. But there are also commonalities in the regions activated during different WM tasks; in particular, there is activity in a fronto-parietal network of regions, with the frontal regions including the middle frontal gyrus, the inferior frontal gyrus and the caudal part of the superior frontal sulcus (Fig. 5B). The intra parietal cortex and caudal superior frontal gyrus are particularly strongly activated in visuospatial WM tasks and in tasks that require spatially selective attention (Rottschy, Langner et al. 2012). Techniques sensitive to the electric discharge of neurons, such as magnetoencephalography (MEG) and EEG, have revealed that during the delay in WM tasks, the activity of frontal regions becomes more synchronous with that in parietal regions (Rottschy, Langner et al. 2012) and that the degree of this synchronicity increases with the amount of information successfully kept in WM. A measure of the overall integration of connectivity into separate networks was found to be positively correlated with WM capacity in healthy adults (Stevens, Tappon et al. 2012).

Another important aspect of WM was the number of items than can be held in attention that is defined ***capacity*** of WM. Working memory capacity (WMC) is used to refer to limited capacity of working memory. The limit of WMC was explained by electrophysiological studies. As more items are added to memory, the total population activity increases, but the activity of inhibitory interneurons also increases. When the number of items added exceeded the capacity, the neural activity representing some of the stimuli decays because of the increased inhibition, and these stimuli cannot be recalled at the end of the delay period. This decay of neural activity corresponds to the limited WMC (Edin, Klingberg et al. 2009).

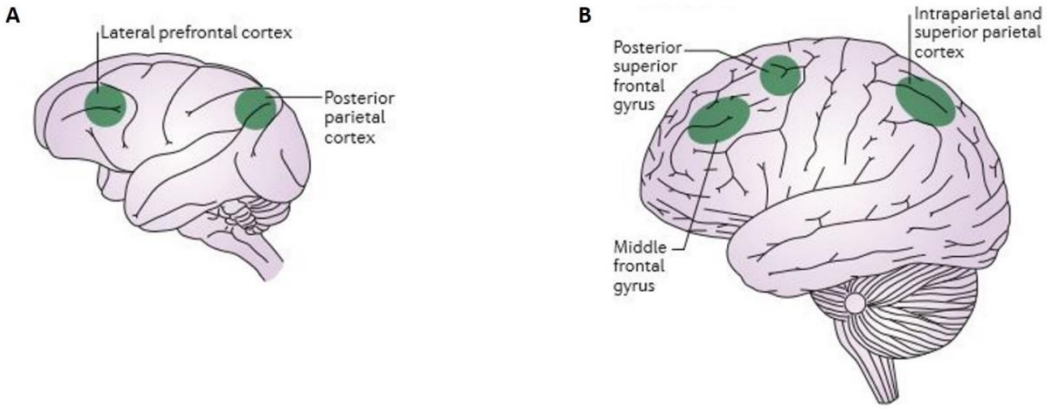


Fig. 5. Neural basis of working memory. *Diagram of the monkey brain, with the areas implicated in visuospatial WM: the posterior parietal cortex and the lateral prefrontal cortex, highlighted in green (**A**). Areas of the human brain activated in visuospatial WM tasks: the intraparietal and superior parietal cortex, the posterior part of the superior frontal gyrus and the middle frontal gyrus (**B**) (Constantinidis and Klingberg 2016).*

Role of the hippocampus in WM

Thus, the common idea about the neural basis of WM is that it is largely mediated by the PFC, and that the HP has little or no role in WM.

Studies in patients with HP damage tested in items WM tasks revealed an involvement of the HP when the number of items to retain in memory was high. Amnesic patients, exposed to an array of 16 toy objects and subsequently tested for object recall, object recognition, and memory for the location of the objects, were impaired in all measures compared to control healthy subjects (Cave and Squire 1991). Amnesic patients with damage limited to HP regions have an impaired recognition memory span performance for line drawings of objects, designs, and odors (Levy, Manns et al. 2003) (Fig. 6).

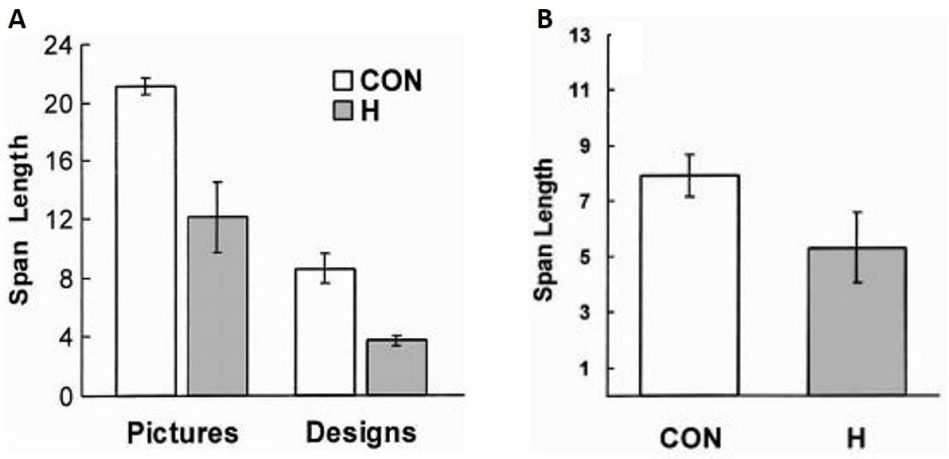


Fig. 6. *Mean visual span length for control volunteers and for amnesic patients with HP damage on two visual span tasks. In one task (left two bars), the stimuli were line drawings of common objects. In the second task (right two bars), the stimuli were kaleidoscope-like colored designs* (**A**). *Olfactory span performance for control volunteers and for amnesic patients with damage thought to be limited to the HP region* (**B**) (*Levy, Manns et al. 2003).*

Oscillatory activity was measured in the HP of patients performing the Sternberg WM task with two classes of stimuli: faces and letters. In this task, participants see a list of 1– 4 stimuli (faces or letters), followed by a 3000 ms delay interval. After the delay, a probe stimulus appears and participants have to indicate whether the probe was a member of the just-presented list. The maintenance interval is defined as the period between 0 and 3000 ms after the offset of the last list item. Stimuli were sets of 16 letters and 16 faces, presented in alternating blocks of 15 lists (Fig. 7 A). An increase of 48 –90 Hz gamma oscillatory power with memory load during the maintenance interval of a WM task was found (van Vugt, Schulze-Bonhage et al. 2010) (Fig. 7 B).

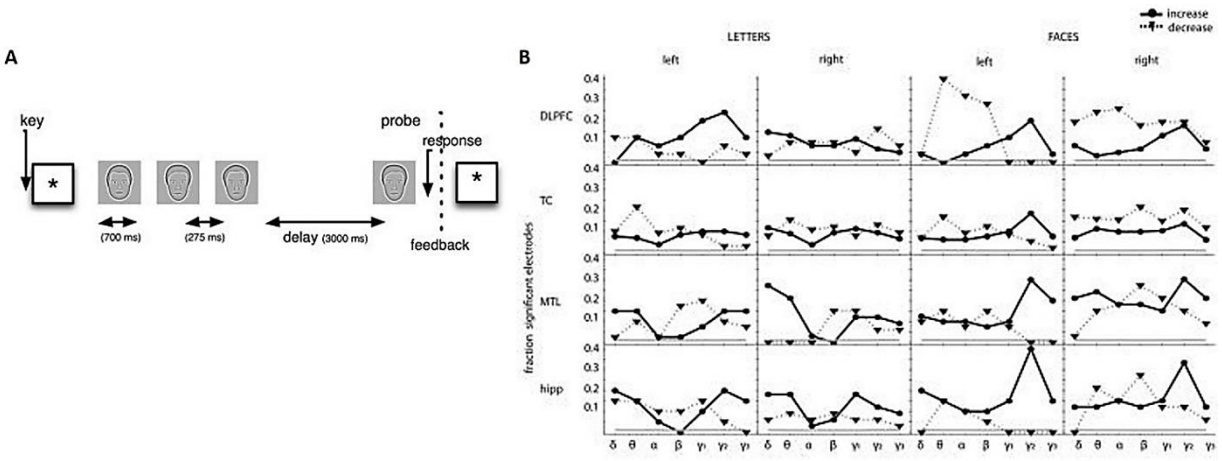


Fig. 7. Brain oscillatory activity in patients performing Stenberg task. *Scheme of the Sternberg task* (***A***). *Fraction of electrodes showing a significant increase (solid) or decrease (dotted) of oscillatory power with memory load. The gray line indicates the fraction of significant electrodes in the permuted data. The frequency bands are as follows: delta (δ), 2– 4 Hz; theta (θ), 4 –9 Hz; alpha (α), 9 –14 Hz; beta (β), 14 –28 Hz; low gamma (γ_1), 28 – 48 Hz; mid-gamma (γ_2), 48 –90 Hz; high gamma (γ_3), 90 –100 Hz. Dorsolateral PFC (DLPFC), Temporal Cortex (TC), Medial Temporal Lobe (MTL) excluded the hippocampus, hippo (hippocampus)* ***(B) (****van Vugt, Schulze- Bonhage et al. 2010).*

Rhesus monkeys with HP lesions were impaired relative to normal control monkeys in a delayed recognition span task which tests the ability to remember an increasing array of stimuli suggesting a role for the HP in WMC (Beason-Held, Rosene et al. 1999). In this task, each trial consists of a series of presentations. In the Spatial condition, a disk is added in a novel position for each presentation. The animal is required to displace the novel disk in each presentation. The span ends when the animal commits the first error. The Color and Object conditions require the animal to displace the novel stimulus in each presentation, but all previously presented stimuli are moved to new positions to preclude the use of spatial cues (Fig. 8 A). HP lesioned monkeys were impaired in all three modalities of the task (Fig. 8 B).

All together these findings in humans and no human primates suggested that the HP is recruited in WM tasks in condition of high memory load.

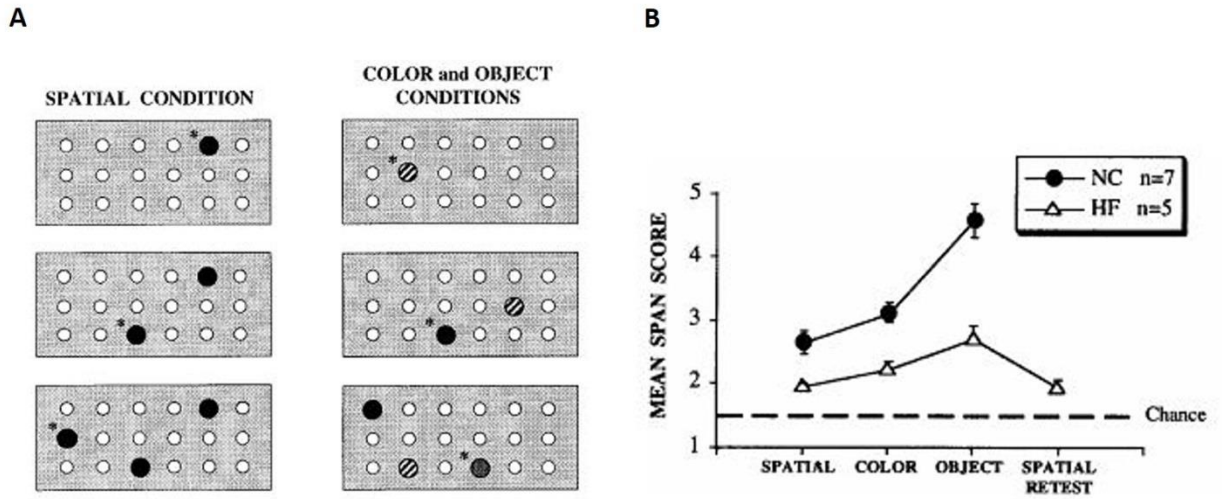


Fig. 8. Scheme of the Delayed recognition span task. *Asterisks indicate the correct response in each of three sequential presentations* (***A***). *Delayed Recognition Span Task performance in HF (HP formation) group relative to control group (NC). HF group is impaired on all modalities of the task relative to control group. Total mean span scores ± SEM.)* (***B***) *(Beason-Held, Rosene et al. 1999).*

WM in rodents

The term WM as applied to animal cognition, originates in the experiments David Olton and Werner Honig in the 1970s. Olton and Samuelson (Olton DS and Samuelson RJ. 1976) devised a classic task for assessing memory in the rodent, the *radial arm maze*. The maze is comprised of eight arms radiating from a central platform. In this maze, the rat is placed on the centre platform, and a food reward is available at the ends of each arm. Olton and Samuelson observed that rats would retrieve food from each arm and quickly learned to visit all the arms without re-entering a previously visited arm. This suggests that rats were able, in a single session, to remember which arms they had visited. This, for Olton et al. (Olton and Papas 1979) was WM: memory that allows the animal to remember which arms it had visited in a session. On the next day, this memory is no longer relevant, as all of the maze arms are baited again.

WM is also defined as a short term memory for an object, stimulus, or location that is used within a testing session, but not typically between sessions (Dudchenko 2004). It is distinguishable from reference memory, which is memory that would typically be acquire with repeated training, and would persist from days to months. Reference memory is often a memory for the rules of a given task, for example, that a bar press produces a food pellet, or that a water maze contains a hidden platform. WM, in contrast, is typically a delay-dependent representation of stimuli that are used to guide behaviour within a task.

Although Olton and Honig were the first researchers to apply the term WM to the animal's short-term storage of information; earlier experimenters had devised tasks for assessing this type of memory in animals. These studies focused on developing tasks to see how long a rat could remember a stimulus that was not present. These were often referred to as *delayed non-matching to sample (DNMS) paradigms*. These tasks required a rat to remember a stimulus over a delay in which that stimulus is no longer present. Following the delay, the rat is presented with the to-be-remembered stimulus and an alternative, and the rat is reinforced for making a response towards the alternative stimulus. Later, a variant of the DNMS task for rodents was introduced, the spontaneous exploration task. This task capitalised on the tendency of rats to explore novel stimuli and environments. Ennaceur and Delacour (Ennaceur and Delacour 1988) developed a task based on the spontaneous exploration of objects. In the task in its simplest form, a rat was presented with an object (A) or pair of identical objects in a small arena. After a brief exposure period, the rat was removed from the arena and a delay ensued. The rat was then brought back to the arena, in which a duplicate of object A and a novel object (B)

are to be found. The rats' natural tendency was to explore the novel object B more than the familiar object A (Fig. 9).

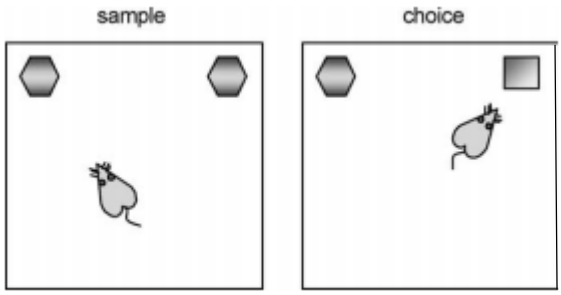


Fig. 9. The spontaneous exploration task of Ennaceur and Delacour (Ennaceur and Delacour 1988). *A rat is presented with an object or pair of identical objects (left plot) which it is allowed to explore. Following a delay, the rat is presented with the previously presented object and a novel object (right plot), and the rat tends to demonstrate its memory for the previously presented object by spending more time exploring the novel object***.**

Radial arm maze: a spatial WM task

Olton and Samuelson (1976) introduced this new spatial paradigm designed to investigate the characteristics of WM. The apparatus was a radial eight-arm maze, with the eight arms radiating away from the central platform like spokes on a wheel. At the beginning of each test, one pellet of food was placed at the end of each arm. Food-deprived rats were placed on the center platform and were allowed to choose freely among the arms until all eight pieces of food were obtained (Fig.10 A). The optimal strategy was to choose each arm once and not repeat choices to any arm. Olton and Samuelson (1976) argued that the animals formed in WM a list of places which had been chosen and which should not be repeated.

This list was developed as animals made their choices, so that, after each correct choice, another item was added to the contents of the WM. WM was described as having the following characteristics: 1) capacity to process accurately at least eight different items; 2) interference among items so that, as the number of places chosen increased, the probability of a correct response decreased; 3) perfect retention for periods up to 1 min.

In the most classic task, described in the pioneering work of Olton and Samuelson (1976), the maze is fully baited and the animal has to visit each arm only once within a trial to find the food reward. Several interesting versions of this task have been developed subsequently.

Jarrard (1983) developed a version of the radial maze to test both WM and Reference memory. In their version of the radial maze, only four of the eight arms were baited with food. The same arms were baited each day and across sessions. The rats learned to not enter in the four non - baited arms. This is the reference component of the task, and an entry into a never- baited arm is considered a reference error, while a re - entry in a baited arm is considered a WM error (Fig. 15 B). Jarrard (Jarrard 1983) also tested rats in a match – to - sample version of the radial maze. In this task, the rat is allowed to explore the maze to find the reward. When it returns in the center of the apparatus is confined there for a delay period. After the delay, the doors of each arm are opened and the rat is required to return in the arm where it obtained the reward.

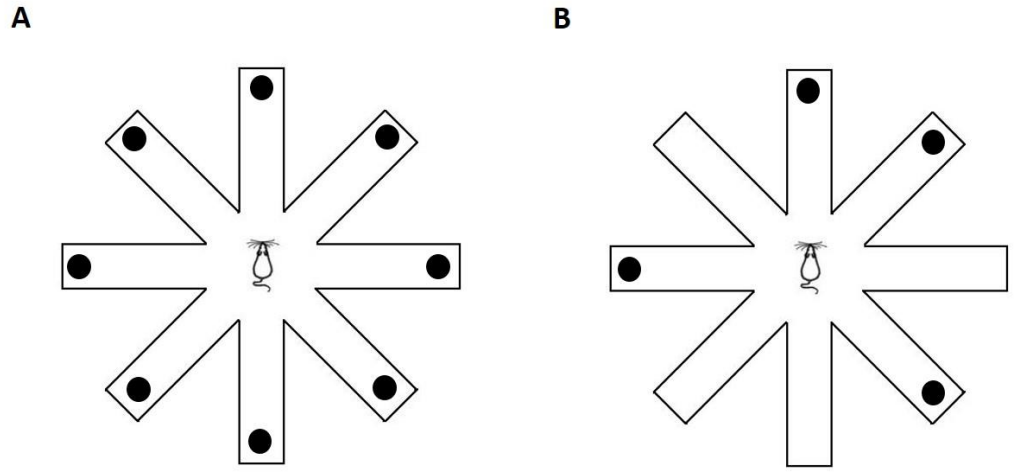


Fig. 10. Scheme of a full-baited radial arm maze task (Olton and Samuelson 1976) *(A)* and a four baited/four unbaited radial arm maze task (Jarrard 1983) *(B)*.

Working memory capacity (WMC) task in rodents

The first WMC tasks in rodents were introduced by Dudchenko et al. (1999). Dudchenko et al. (Dudchenko, Wood et al. 2000) trained rats on an odor span task. Rats were first presented with a cup of sand scented with a household spice. A food reward was obtained by digging in this cup. After a short delay, the rat was again presented with a cup of sand scented with the first odour, and a cup of sand scented with a novel odour. Reward was only available in the cup of sand with the novel odour. After another delay, the rat was presented with three cups of sand, two of which had been previously presented, and one which contained a novel odour. As before, reward was only available in the cup contained the novel odour. The task continued until an error was committed (Fig. 11).

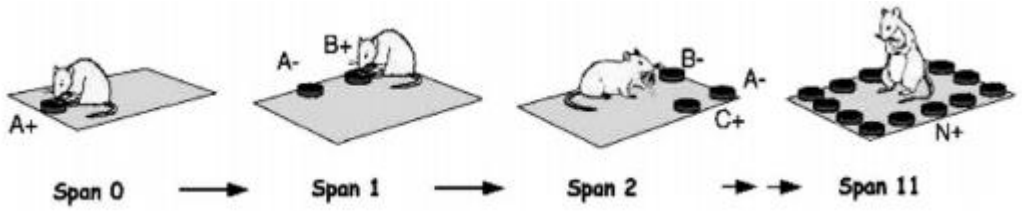


Fig. 11. Odour 'span' task. *A rat is first presented with a cup of sand that is scented with a specific odour* (***A+***)**.** *After a delay, the rat is presented with a cup of sand scented with that same odour, and one scented with a different odour* (***B+***)**.** *Food reward is available only in the cup containing the non-matching odour (**B+**). Additional cups of sand, scented with different odours, are presented to the rat and its task is to remember which scents it has previously sampled. The rat's span is the number of odours it can correctly remember before making an error (Dudchenko, Wood et al. 2000).*

A spatial span task was also developed to explore the contribution of the HP to performance in a spatial version of the span task (Dudchenko, Wood et al. 2000). This task was similar to the odour NMS task, but here animals were required to remember which location it had visited, and then to select a different choice location. Animals were first presented with a cup of unscented sand in one of 21 locations along the platform perimeter. After digging in the sand, the animal was removed from the platform and placed in an opaque bucket. The experimenter then replaced the sample cup with another cup of unscented sand in the same location and placed an additional cup in a new randomly selected location on the platform. Reinforcement was available only in the cup in the new location. Thus, after the animal made a correct response on the initial choice phase of the task, it was placed back in the opaque bucket, and an additional cup with unscented sand was added to the platform in a new location. The remaining cups on the platform were replaced as well, so that the animal could not solve the task by marking the cups. When returned to the platform, the animal was required to dig in the cup at the new location to obtain another buried reward. A cereal reward was buried in the sand of this cup. If the animal made a correct response, it was put back in the bucket, and yet another cup was added at a new location. If the animal dug in the cup at a previously presented location, the trial was terminated (Fig.12).

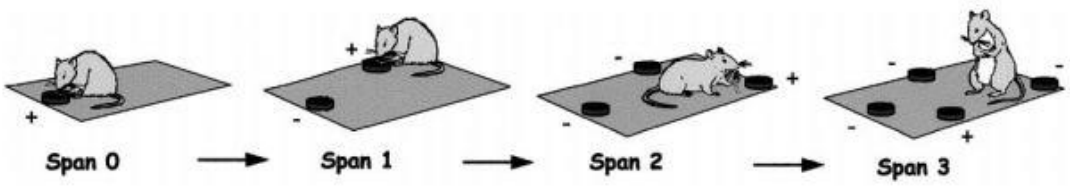


Fig. 12. Schematic of the spatial span task. *An animal was presented with an unscented cup of sand in a specific location on the platform. After retrieving the buried reward, the animal was removed from the platform, and a second cup of sand was added in a different location. The animal's task, on its return to the platform, was to remember the location of the cup that it had already sampled and select the cup in the new location. Additional cups in additional locations were presented in the same manner (Dudchenko, Wood et al. 2000).*

Different objects recognition task: an object WMC task in mice

A modified form of the spontaneous object recognition task was developed to assess object WMC in mice (Sannino, Russo et al. 2012). In this version, the memory load was augmented by increasing the number of objects to which mice were exposed (3, 4, 6 or 9 Different Objects Task, DOT). As control task, an Identical Objects Task (IOT) was used in which was increased the number of identical objects, which is supposed to not increase the amount of information to be processed (Fig. 13). The DOT consists of a study phase during which the animals are free to explore the objects for a maximum of 10 or 15 min depending on the number of objects to explore. After a delay of 1 min, the test phase starts. During the test phase, the animals are exposed to identical copies of familiar objects and a new object. The IOT consists of the same phases except that 35 sec of total exploration are allowed in a 5 min test independently of the number of items. The index of new object discrimination used is the time spent exploring the new object compared the familiar objects. New object discrimination is considered to occur when the new object was explored significantly more than all of the other familiar objects present during testing. Exploration was defined as the time in which the nose was in contact (< 2 cm from the object) with the object (Broadbent, Squire et al. 2004).

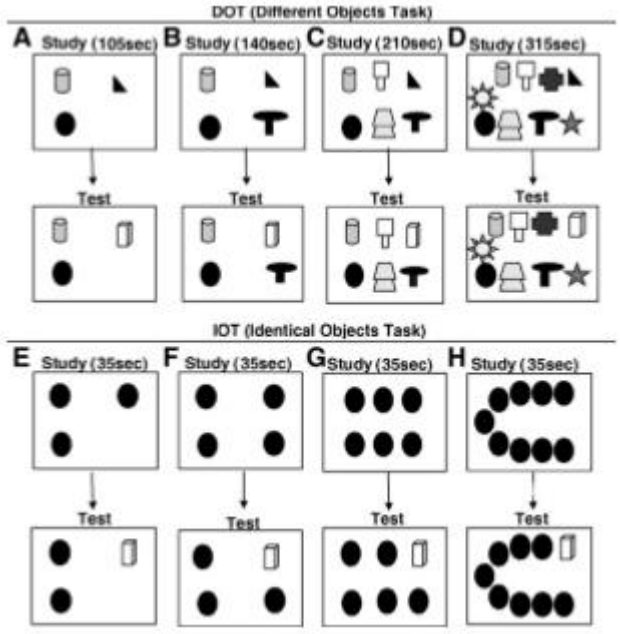


Fig. 13. Schematic representation of the study and test phase of the different/ identical objects task. *During the study phase, animals are exposed to the* (***A–D***) *3-DOT, 4-DOT, 6-DOT, and 9-DOT (Different Objects Task) objects or* (***E–H***) *IOT (Identical Objects Task) objects. The figure reports the object exploration time (in seconds) the animals are allowed to accumulate during the study phase. During the test phase, only one object is substituted with a different one in all conditions (Sannino, Russo et al.2012).*

Radial arm maze: a WMC version of the task

First Olton (1977) studied WMC on a 17 arms radial arm maze, then Tarantino et al. (2011) used a 12 arms radial am maze to assess WMC. A different WMC version of the radial arm maze task was introduced in my laboratory to assess the contribution of the information load on spatial memory (Olivito, Saccone et al. 2016). The WM load was increased by increasing the number of open and baited arms. The test consists of a ***habituation phase*** that habituates the animals to the apparatus and to the food pellet scattered through it (Day 1-Day 3). In the ***pre-training phase*** (Day 4-Day 5) only two arms are open and baited. This phase habituates the animals to the mechanism of the task that is to enter once in each arm (Fig.14 A).

The WM load was then manipulated by modifying the number of open and baited arms. In the ***WM training phase*** the trials varied from 3, 6 and 8 open and baited arms (Fig.14 B). In the last phase, called ***reference memory (RM) training phase*** (Day 15– Day 16), all eight arms were always open and the baited arms were kept constant across trials and across days, so that animals could rely on RM to select them. An RM error was codified as entering in an unbaited arm. A WM error was the re-entry in a baited arm (Fig.14 C).

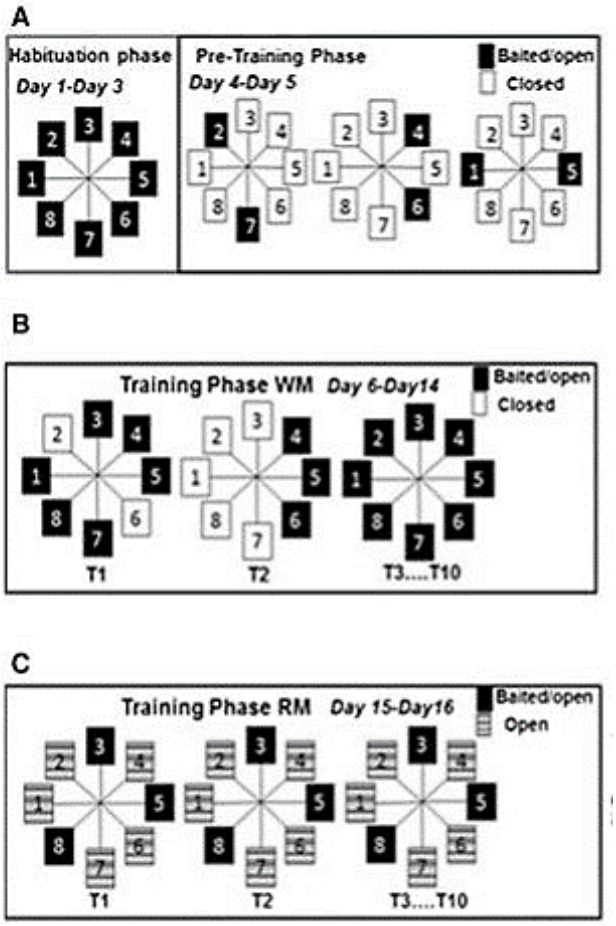


Fig. 14. Schematic representation of a WMC radial arm maze task. *In the habituation phase (Day 1-Day 3) the animals explore the maze for 10 min (**A**). In the pre-training phase (Day 4-Day 5) only two arms are open and baited (**A**). In the WM training phase (Day 6-Day 14) the number of open arms switches between 3,6 and 8 open arms (**B**). In the RM training phase (Day 15-Day 16) all arms were open but only 3 of them were baited (**C**) (Olivito, Saccone et al. 2016).*

Spatial memory: the role of the hippocampus

Research about the role of the HP in memory has earned much interest following the discovery of ***place cells*** in rats (O' Keefe and Dostrovsky 1971). In 1971, O'Keefe and Dostrovsky found that single HP neurons increased their firing rate whenever a rat traversed a particular region of a chamber (O' Keefe and Dostrovsky 1971). When they recorded extracellular action potentials from the HP of freely moving rats, some HP pyramidal cells demonstrated firing patterns that seemed to depend on the animal's location in the environment. When the rat left the "place" that was encoded by a given cell, the cell fell almost silent. In an open field, the firing rate was also independent of the direction in which the animal entered the area and the direction that it was facing. The firing of each cell seemed to indicate a specific location in the environment of the rat; so these cells are called "place cells". Since 1971, neuroscientists have conducted hundreds of studies to characterize the properties and

dependencies of place-cell activity. Studies using freely behaving rats have shown that pyramidal cells show stable, long-lasting, environmentally specific place fields, with between 30% and 50% of the CA1 cell layer showing place-specific activity in any given environment (Thompson and Best 1989). When an animal is introduced to a novel environment, these place cells form rapidly, usually within five minutes, and are maintained robustly (Wilson and McNaughton 1993). Place cells have been best characterized in area CA1 of the rat HP, but they have also been studied in the other HP subfields and in the entorhinal cortex (Quirk, Muller et al. 1992). These cells, located in specific subregions of the HP, respond to different environment and the features within them. Collectively, they form a neural map of the environment, and these cells remap the environment as the organism moves within a given space or moves to a new space. In recent years, the role of the entorhinal cortex in spatial mapping has been elucidated (Leutgeb, Leutgeb et al. 2005). Place cells fire when an animal is at specific locations in an environment and, as the animal explores an open environment, the ensemble of cells provides a stable representation of the animal's location, independent of its orientation. The place fields are maintained even when all orienting cues are removed, demonstrating that they encode the 'memory' of a location. Place fields in familiar environments remain stable for several weeks, which suggests that place cells encode a long-term memory for that environment (Lever, Wills et al. 2002). Place cell representation remains stable to minor changes in the environment. If a familiar environment is dramatically changed, the place-cell representation will abruptly change (referred to as 'remapping')(Bostock, Muller et al. 1991) to signal that the animal is now in a different environment. A crucial role for the HP in the retrieval of events has been proposed with place cells reactivating representations of the spatial geometry of the environment and the locations of objects in it. In presence of a cue, a place cell will reactive and, through a process of pattern completion, will allow to retrieve the surrounding environmental features that are bound to (that is, perceivable from) that location.

Thus, place cell firing does not simply reflect direct sensory inputs; if that was the case, the firing would change greatly as the rat changed the direction in which it was facing. Besides responding to incoming perceptual information, place cells are also driven by self-motion signals (proprioceptive, vestibular and reafferent signals from intended movements), which indicate the location of the animal on the basis of its own movements — a process referred to as 'path integration'. This process is probably supported by so called **"grid cells"**, which have a strikingly regular spatial-firing pattern and are found in the entorhinal cortex (Hafting, Fyhn et al. 2005), the main neocortical input to the HP. When electrodes were placed directly in the medial part of the entorhinal cortex was found that many neurons were as sharply modulated by position as place cells in the HP. The multiple firing fields of individual entorhinal neurons formed a regularly spaced triangular or hexagonal grid pattern,

which repeated itself across the entire available space. Grid cells are organized in a non-topographic manner, much like place cells. The discovery of grid cells was followed by studies showing that these cells were part of a wider spatial network comprising other cell types as well, such as *head direction –modulated cells* (Sargolini, Fyhn et al. 2006) and cells that fire specifically along one or several borders of the local environment (*border cells*). (Savelli, Yoganarasimha et al. 2008) (Fig. 15 A-B). Head direction cells had previously been observed in a number of brain systems, from the dorsal tegmental nucleus in the brain stem to the pre- and parasubiculum in the parahippocampal cortex (Taube, Muller et al. 1990). First discovered in the presubiculum, head direction cells are found in other regions as well, including in the entorhinal cortex, thalamus, mammillary nucleus, retrosplenial cortex, and dorsal striatum. They discharge in relation to the animal's directional heading with respect to the environment in the horizontal (yaw) plane. As the place cells and the grid cells they use the surrounding environment as its reference frame, they are classified as allocentric (Taube and Muller 1998). Recent studies report that head direction cells are especially important for egocentric navigation. Allocentric and egocentric navigation are two distinct types of navigation. Allocentric navigation, also referred to as spatial navigation, is characterized by the ability to navigate using distal cues, that is, cues/landmarks located outside and at some distance from the organism. Egocentric navigation is characterized by the ability to navigate using internal cues, that is, feedback from limb movements for rate of movement (speed), directions, turns and sequence of turns. Egocentric navigation can operate in darkness, indicating that visual cues are not essential for this method of navigating, although in the absence of visual cues egocentric navigational accuracy is reduced. By contrast, allocentric navigation is disabled by the absence of visual cues. Egocentric navigation generally refers to the ability to navigate by internal self-movement cues, but a further distinction can be made by dividing egocentric navigation into *route-based* and *path integration* (Etienne and Mauren 1996). Route-based navigation relies on internal cues of rate of movement, turns, whereas path integration relies on these but involves an additional attribute, vector addition. In route-based navigation, an organism follows a path with the order of turns remembered as a set of specific rules, such as straight-left-right-left-left, that is which direction to turn when it reaches specific signposts o moves in a specific direction for a given number of steps. Interestingly, these memorized operations can be overlearned to such a degree they become habits. When this occurs, the location of memory shifts within the brain and is reclassified as implicit or procedural memory. In humans, implicit memory is seen as skilled behaviour that is relatively automatic and not conscious, such as the sequence of movements involved in driving a car, riding a bicycle, hitting a baseball, skiing and so on. By contrast, path integration is seen as the ability of an organism to leave its home-base and move to different locations and then return by a different, more direct path. For example, an organism could

travel from its home (H) to location A, location B, and location C and then return to its base by a more direct path from C to H without retracing its steps in reverse through B and A.

Different brain regions mediate allocentric and egocentric spatial memory. Interestingly, a recent fMRI study on human subjects revealed that different mainly right hemispheric networks are involved when egocentric (fronto-parietal) or allocentric spatial judgments (frontal and HP) on intersecting horizontal and vertical bars were given (Galati, Lobel et al. 2000). The subjects had to judge the position of a vertical bar that could appear at 4 positions left and right of the center of the projection screen. A larger horizontal background bar was drawn behind the vertical target bar. Subjects were required to judge either the position of the vertical bar with respect to the body midline in egocentric task blocks or the position of the vertical bar with respect to the center of the horizontal background bar in allocentric task blocks. As expected, the parietal lobule was more active during egocentric spatial judgments (Medendorp, Goltz et al. 2005), whereas superior temporal cortex and areas around the HP/HP formation were more active during allocentric judgments (Galati et al., 2000). Areas holding allocentric representations of space are thought to subserve the conscious perception of objects, or memory functions, and are found mainly along the ventral processing stream (Goodale and Milner, 1992). In particular, the brain regions crucial to mediating allocentric navigation are the HP and entorhinal cortex. The HP has been identified for many decades as a key structure in forming cognitive maps (O' Keefe and Nadel, 1978). Lesions, pharmacological inhibition, loss-of-function genetic mutations of signalling molecules or receptors within the HP result in impaired spatial learning and memory (Brandeis, Brandys et al. 1989). Egocentric representations can be found, on the contrary in dorsal stream brain areas subserving goal-directed actions (Goodale and Milner 1992).

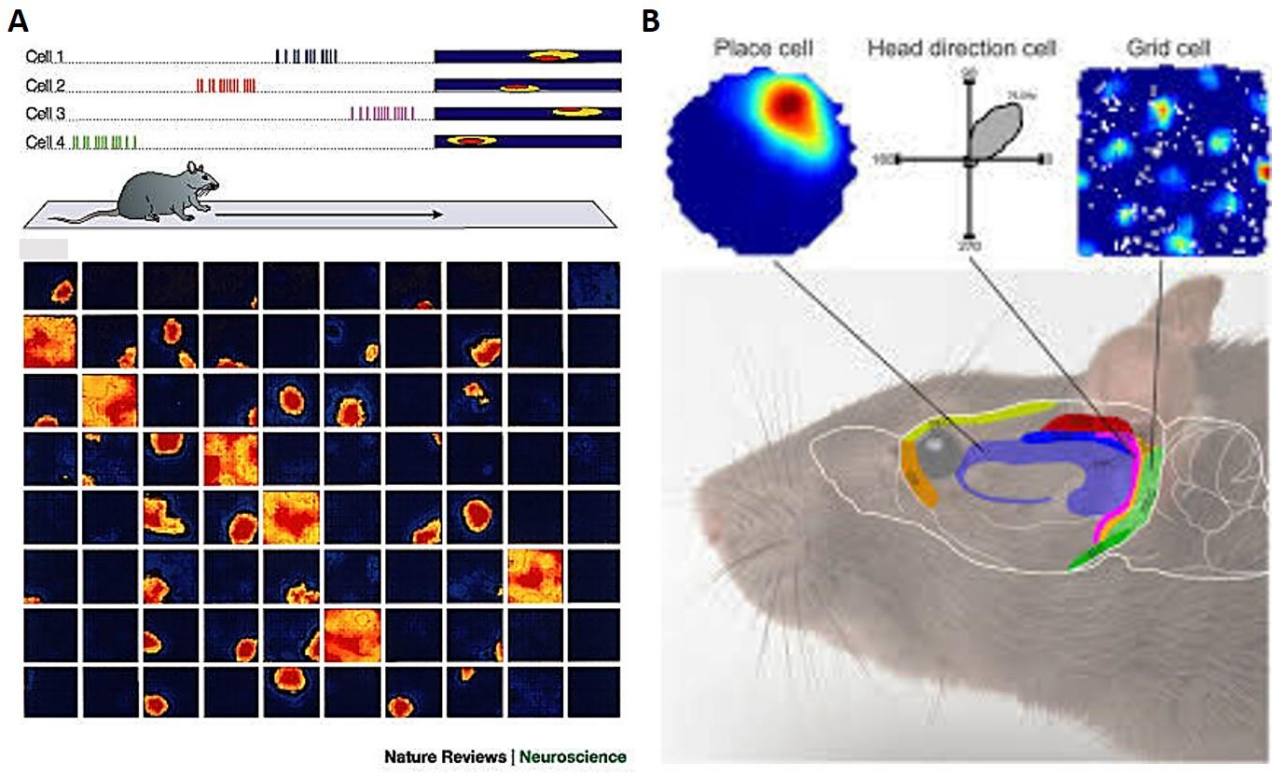


Fig. 15. *Place-specific firing properties of HP pyramidal cells as a rodent runs down a linear track. A common method of representing these place fields (right) is a firing rate map with areas of high firing rate coloured red and yellow and areas with no firing coloured blue. In the bottom, there are the firing rate maps of cells simultaneously recorded from area CA1 of a rat exploring a square arena (Nakazawa et al., 2004)* (**A**)**.** *Three neurons are thought to form the cognitive map of the space: place, head direction cells and grid cells (Grieves and Jeffery 2017)* (**B**)**.**

An example of egocentric spatial memory in the radial arm maze task

Superimposed on spatial, associative, tactile, olfactory or other modes of information processing that may contribute in parallel to place learning, it is often possible to observe regular patterns in the way animals search for safe or rewarded locations. In constrained mazes, such as the radial arm maze, certain individual search strategies can be identified.

The eight arms- radial arm maze has been used extensively in studies of spatial memory in animals but egocentric strategies, that is subjects repeatedly choosing arms a fixed distance apart particularly adjacent arms, are so prominent. The prevalence of egocentric strategies raises possibility, but does not prove that egocentric strategies may play a major role in radial maze performance. Although numerous experiments have shown that subjects can solve the radial maze problem using memory for sets of extramaze stimuli, there are situations in which response patterns appear to be important for the accurate choice of arms. Some animals always enter adjacent arms, particularly in mazes

without doors, others choose alternating or opposite arms. Sequential arm entry is a highly efficient strategy in working-memory tasks with all arms baited (Olton, Becker et al. 1980) but not in partially rewarded mazes. Egocentric strategies exhibited by the animals are not a rule. They appeared to represent a secondary egocentric strategy superimposed upon a primary dependence on environmental or movement-generated cues. Subjects may exhibit egocentric strategies because they are foraging efficiently, obtaining all the reward after travelling the least possible distance or in the least possible time. According to this point of view, egocentric strategies should develop only after a subject has learned to solve the radial maze problem by other means. On the other hand, egocentric strategies may have occurred on the free-choice trials because subjects were using movement-generated stimuli to guide their choices on such trials. Rats are sensitive to the directions they have travelled on a maze and vestibular cues can be important to spatial orientation.

Egocentric strategies or turning responses were defined in terms of the angle between successively chosen arms. Rats frequently employed the 45° turning responses in the first training block, but this rapidly diminished as training progressed (Okaichi and Oshima 1990).

This systematic exploratory behaviour is considered to be an innate behaviour rather than an acquired one because it appears at a high rate after the rats have performed three to four trials. When all arms are baited, rats continue to use this strategy as the best strategy (Bolhouis, Bijlsma et al., 1986,Crusio, Schwegler et al. 1987). When the egocentric strategy is not the best strategy, as in this experiment, rats shift to the most efficient strategy as training progresses.

The changes in the choice behaviour of controls confirm previous suggestions that the egocentric strategy is flexible in nature (Olton, 1979) and that rats can utilize the most effective strategy when more than one strategy is simultaneously available (Dale and Innis, 1986).

Animals' strategies were also evaluated in terms of degree of divergence. Within a trial, all the transitions between two consecutive arm choices are quantified by counting one unit of divergence when the rat went successively in two adjacent arms, by counting two units of divergence when the rat went successively into arms separated by one arm, by counting three units of divergence when the rat went successively into arms separated by two arms, and so on. A particular exploratory behaviour, called clockwise strategy, has been observed in rodents (Olton 1977). Clockwise serial searching strategy consists of visiting adjacent arms systematically in a clockwise or counterclockwise direction. In this case, the degree of divergence equals 7.

Rats tested in a no-confinement procedure of the radial arm maze exhibited a degree of divergence equal to 7 or a predominance of a 45° angle. In contrast, in the 10 seconds confinement procedure,

rats never exhibited degree of divergence equal to 7. The no-confinement and the confinement procedure are two different procedures of the radial arm maze task. The no-confinement procedure defines a procedure of the radial arm maze task where the animals are free to explore the maze. In the confinement procedure the animals are confined in the central zone of the maze between arm entries in order to prevent them to develop egocentric strategies. Rats tested in a confinement procedure and subsequently in a no-confinement procedure (test day) performed the task with a predominance of 45° angles in the no-confinement procedure, demonstrating that this stereotypic behaviour is used whenever possible, even by rats that had learned the task without this strategy (Fig. 16). The tendency to perform the task with clockwise strategy is a spontaneous behaviour (Pico and Davis 1984). As suggested by some authors (Yoerg and Kamil, 1982), this spontaneous behaviour fits with the concept of optimal foraging. Although this behaviour is spontaneous, it did not occur on the first day that no errors were made, but it was adopted across sessions to enable rats to obtain a better performance.

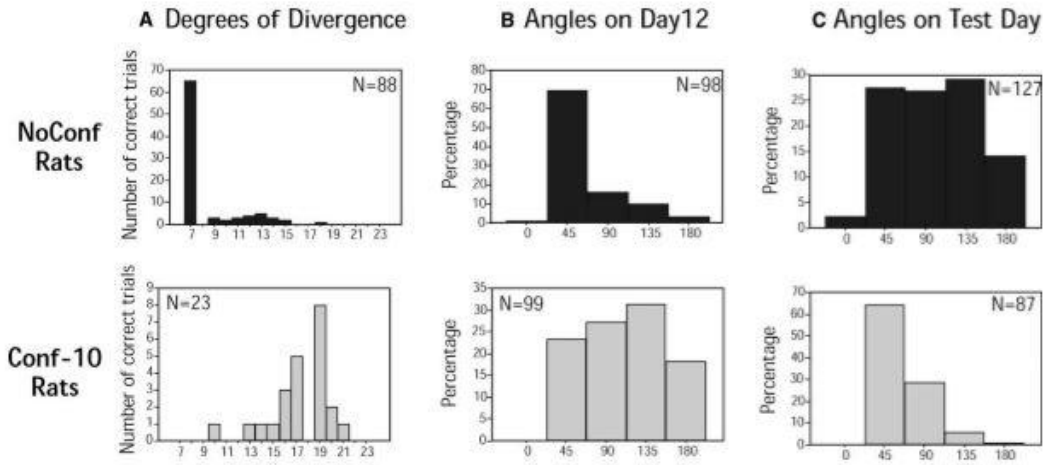


Fig. 16. *Analyses of the strategies for the rats trained in the No-Confinement (NoConf) condition (top panels), and for the rats trained in the 10 seconds Confinement (Conf-10) condition (bottom panels) (Dubreuil et al., 2003).*

Anatomical differences within the hippocampal formation: a ventral and a dorsal hippocampal region

The different cognitive operations, and mnemonic functions of the HP formation might derive from the fact that HP is not a homogenous region. Two distinct regions are segregated along the dorso-ventral HP axis: the dorsal and the ventral HP (Strange and Witter 2014).

Anatomic studies reported that afferent inputs from entorhinal cortex to dorsal and ventral HP are different. The entire entorhinal cortex can be divided into three relatively independent band-like zones: the caudolateral, intermediate, and rostromedial zones. In general, the caudolateral band receives the most visuospatial information (mostly via adjacent perirhinal and postrhinal cortex), and in turn, projects specifically to the dorsal/septal (caudal in monkey) HP region. The medial band, which receives primarily olfactory, visceral, and gustatory inputs, projects specifically to the ventral/temporal (anterior in monkey) HP; while the intermediate band seems to receive even more widespread inputs and projects primarily to the intermediate parts of the HP (Burwell 2000).

Moreover, the dorsal (septal, caudal in primates) CA1, which contains the greatest density and selectivity of place cells coding spatial location (Jung et al., 1994) sends excitatory projections to the dorsal parts of the subiculum, presubiculum, and postsubiculum (Amaral, Dolorfo et al. 1991). The dorsal parts of the subicular complex contain mainly “head direction cells” for coding head position in space. The most prominent cortical projections from the dorsal CA1 and the dorsal parts of the subicular complex are to the retrosplenial and anterior cingulated cortices (Cenquizca and Swanson 2007). These are two cortical regions involved primarily in the cognitive processing of visuospatial information and memory processing and environmental exploration. Meanwhile, the dorsal parts of this subicular complex send massive parallel projections through the postcommissural fornix to the medial and lateral mammillary nuclei and the anterior thalamic complex, two structures containing the most navigation-related neurons. Additionally, the dorsal CA1 and dorsal CA3 project rather selectively to the caudal part and dorsal region of the medial zone of the rostral part of the lateral septal nucleus, which in turn projects to the medial septal complex and supramammillary nucleus, two structures that generate and control the HP theta rhythm activated during voluntary locomotion. Finally, the dorsal subiculum and lateral band of the lateral and medial entorhinal cortex send massive projections to the rostrolateral part of the nucleus accumbens and rostral caudoputamen, both of which send descending projections to the ventral tegmental area and/or reticular part of the substantial nigra. These structures, together with the immediately adjacent mammillary body in the caudal

hypothalamus control exploratory or foraging behaviour. In short, the dorsal HP-subiculum complex forms a critical cortical network with the retrosplenial and anterior cingulate cortical areas that mediate cognitive process such as learning, memory, navigation, and exploration (Fig.17 A).

About the ventral HP connectivity, the first distinct connectivity of ventral CA1 from that of dorsal CA1 is in its direct projection to the olfactory bulb and several other primary olfactory cortical areas, including the anterior olfactory nucleus, piriform cortex, and endopiriform nucleus. Such projections may play a role in the depression-like symptoms that follow loss of the olfactory bulb. Next, the ventral CA1 and ventral subiculum share massive bi-directional connectivity with amygdalar nuclei that receive main and accessory olfactory sensory inputs. Additionally, the ventral CA1/subiculum and these amygdalar nuclei also share intimate bi-directional connectivity with the infralimbic, prelimbic and agranular insular cortices. These ventral HP/subicular-amygdalar-medial prefrontal cortical structures form a series of parallel, segregated descending projections, either directly or indirectly through the lateral septum and bed nuclei of the stria terminalis (BST) to innervate the periventricular and medial zones of the hypothalamus, the primary structure involved in the control of neuroendocrine, autonomic, and somatic motor activities associated with three basic classes of motivated behaviors having strong emotional components: ingestion, reproduction and defense (Dong, Petrovich et al. 2001a). Projections from the ventral HP to the anteromedial group of the BST may be critical for understanding neuroendocrine dysfunctions associated with psychiatric disorders (such as depression, anxiety, and PTSD). The BST is one critical relay station for the HP regulation of the hypothalamic-pituitary-adrenal response to psychological stress and plays an important role in anxiety. Second, the direct projections to the central amygdalar nucleus is responsible of the role of the ventral HP in mediating fear learning (Maren and Holt 2004).

It is worth noting that the ventral CA1, along with the ventral subiculum and medial band of the lateral and medial entorhinal cortical areas, also gives rise to direct projections to the caudomedial (shell) nucleus accumbens which plays a critical role in reward processing and motivation of feeding behaviour. In summary, the connectivity of the ventral HP places it in an ideal situation to regulate the impact of emotional experiences and to control general affective states (Fig. 17 B).

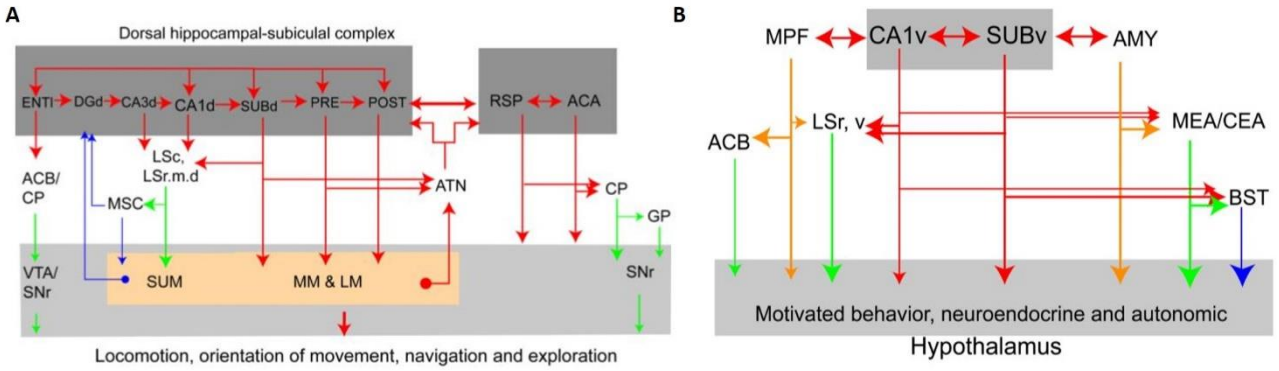


Fig. 17. *Dorsal HP connectivity.* (*Abbreviations: ACA, anterior cingulated area; ACB, nucleus accumbens; ATN, anterior thalamic complex; CP, caudoputamen; DGd, dorsal domain of the dentate gyrus; ENTl, the caudolateral band of the entorhinal cortex; GP, globus pallidus; LM, lateral mammilary nucleus; LSc, the caudal part of the lateral septal nucleus; MM, medial mammilary nucleus; MSC, medial septal complex; PRE, presubiculum; POST, postsubiculum; RSP, retrosplenial cortex; SNr, reticular part of the substantial nigra; SUBd, dorsal subiculum; SUM, supramammillary nucleus; VTA, ventral tegmental area)* (**A**)**.** *Ventral HP connectivity. (Abbreviations: ACB, nucleus accumbens; AMY, cortical-like amygdalar areas (nuclei); BST, bed nuclei of the stria terminalis; CEA, central amygdalar nucleus; LSr, v, the rostral and ventral parts of the lateral septal nucleus; MEA, medial amygdalar nucleus; MPF, medial prefrontal cortex; SUBv, the ventral subiculum) (**B**) (Fanselow and Dong 2009).*

A recent view of the HP connectivity along its dorso-ventral axis reported that it is gradual rather than absolute which suggests that functional differences along the long axis may also exhibit a gradient-like organization. A dorsolateral-to ventromedial gradient of origin in the entorhinal cortex (EC) corresponds to a dorso-ventral axis of termination in the HP. For example, the infralimbic and prelimbic portions of the cingulate cortex primarily reach ventral parts of the HP via input to the ventromedial parts of the EC. The remaining parts of the cingulate cortex — anterior cingulate and retrosplenial cortices — primarily target dorsal and lateral parts of the EC, which subsequently project to dorsal parts of the HP. The HP thus receives a transition of projections from the cingulate cortex along its long axis: cingulate areas involved in emotional regulation (infralimbic and prelimbic cortices) project to more ventral regions, and cingulate areas involved in spatial processing (the retrosplenial cortex) project to more dorsal regions. In the same way, HP connectivity with multiple subcortical structures also shows dorso-ventral topographical gradients (Fig.18 A). Taking the topography of the major HP output to the lateral septum (LS) as an example, the dorsal half of the HP projects to a very small dorsal part of the LS, whereas progressively more ventral parts of the HP innervate progressively larger parts of the LS more ventrally. Crucially, this topographically graded organization is preserved in LS projections to the hypothalamus (Fig.18 B). This implies that different HP regions along the longitudinal axis topographically map onto different hypothalamic regions. HP connectivity with the nucleus accumbens (NAc) and amygdala also follows a topographical pattern,

with progressively more ventral HP portions projecting to progressively more medial parts of both of these subcortical structures (Fig. 18 C).

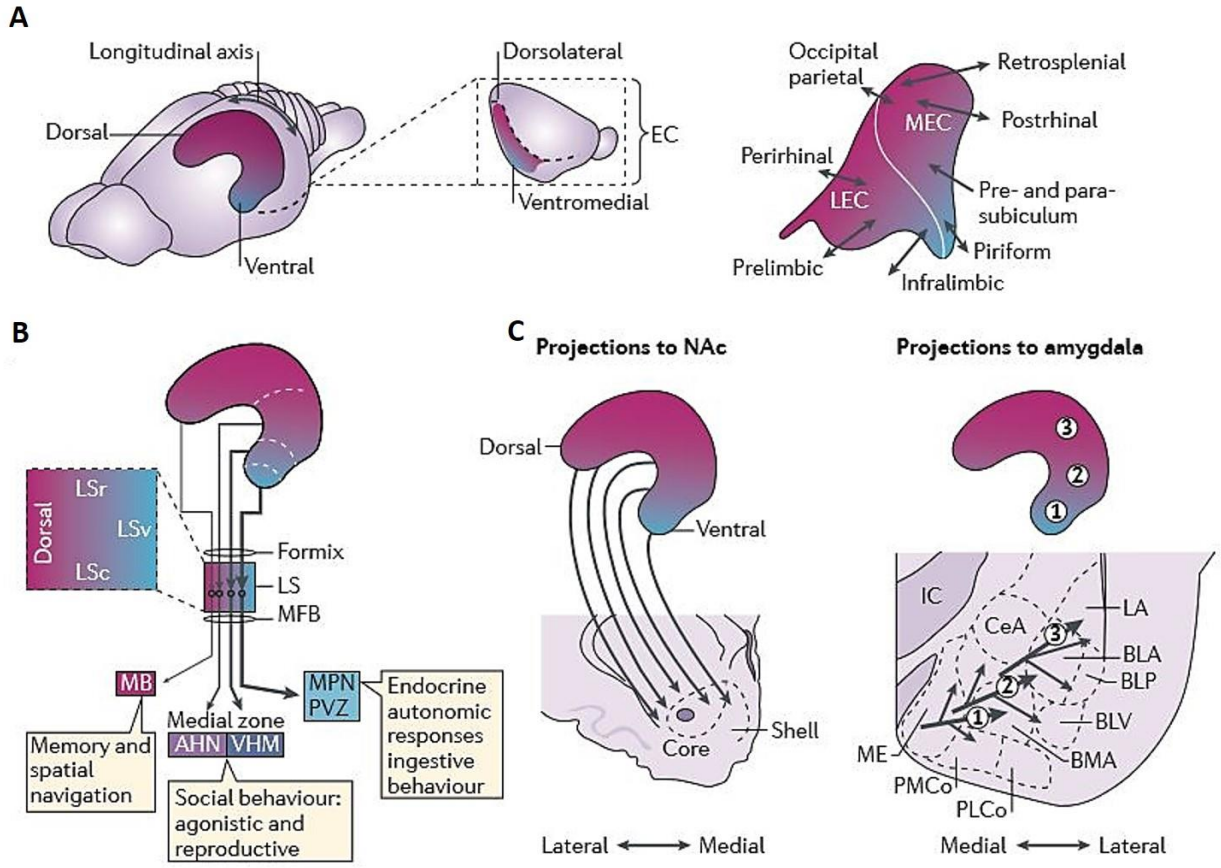


Fig. 18. Extrinsic connectivity gradients. *A representation of the topographical arrangement of entorhinal–HP reciprocal connections in rodents (**A**). The HP output to the lateral septum (LS) and hypothalamus. The most ventral tip of the CA1–subiculum (blue) projects to LSv, which projects to the medial preoptic nucleus (MPN) and hypothalamic periventricular zone (PVZ). More dorsal parts of the CA1–subiculum field project to the LSr, which in turn projects to hypothalamic medial zone nuclei, including the anterior hypothalamic nucleus (AHN) and the ventromedial hypothalamic nucleus (VMH). The dorsal subiculum sends a small projection to the dorsal LS, which is relayed to the mammillary body (MB) (**B**). Topographical gradient of projections from the HP to the medial (shell)-to-lateral (core) portions of the nucleus accumbens (NAc) and the medial-to-lateral portions of the amygdala. Note the absence of projections from the dorsal HP and the relative lack of innervation of the central nucleus of the amygdala (CeA). BLA, basolateral amygdala; BLP, posterior basolateral nucleus of the amygdala; BLV, ventral basolateral nucleus of the amygdala; BMA, basomedial nucleus of the amygdala; IC, internal capsule; LA, lateral amygdala; ME, medial nucleus of the amygdala; MFB, medial forebrain bundle; PLCo, posterolateral cortical nucleus of the amygdala; PMCo, posteromedial cortical nucleus of the amygdala (**C**) (Strange, Witter et al. 2014).*

Functional differences were also found in several behavioral studies. A big portion of literature considers the HP as a purely cognitive structure involved in memory, while there is a consistent part of research that considers it as a regulator of emotion whose dysfunction lead to affective disorders,

such as depression. However, gene expression and anatomical projections patterns that vary along the rostral/caudal-dorsal/ventral extent of the HP suggest that it can be divided into separate structures: a dorsal and a ventral subregion in rodents corresponding to a posterior and an anterior subregion in humans, respectively (Fig. 19 A) (Strange, Witter et al. 2014). The HP can be thought as a set of separate structures with a rostral/dorsal zone that serves the cold cognitive function and a caudal/ventral zone that corresponds to the hot/ affective HP. Moser and Moser 1998 (Moser and Moser 1998) suggested that the HP may not act as a unitary structure with the dorsal (septal pole) and ventral (temporal pole) portions taking on different roles.

Their argument was based on 3 data sets. First, prior anatomical studies indicated that the input and output connections of the dorsal HP and ventral HP are distinct (Swanson and Cowan, 1979). Second, spatial memory appears to depend on dorsal HP not ventral HP (Moser, Moser et al. 1995). Third, ventral HP, but not dorsal HP, lesions alter stress responses and emotional behavior (Henke 1990).

Behavioural tasks as the Morris water maze task have been particularly informative regarding to this distinction. The Morris water maze task clearly implicates the dorsal HP in spatial memory. Lesions restricted to as little as 25% of the dorsal HP impair acquisition on the water maze. Lesion restricted to the ventral HP have no effect on this behaviour (Moser, Moser et al. 1995). Consistent with the lesion data, there is a greater density of place fields in the dorsal HP as opposed to ventral HP (Jung, Wiener et al. 1994). A local cluster of place cells in the rodent dorsal HP can cover most of a spatial environment. Initial evidence suggested that relatively small segments of the dorsal HP (a quarter or less of total HP volume) are sufficient to encode spatial memory. On the contrary, the proportion of ventral HP cells that express place fields were markedly lower than that of dorsal HP cells expressing place fields and that ventral place cells have lower spatial selectivity.

In a study that clearly manipulated stress over cognition, Henke (1990) reported that ventral HP but not dorsal HP lesions enhanced cold/restraint stress ulcers. Furthermore, Kjelstrup et al (2002) reported that lesions of the most ventral quarter of the HP increased entry into the open (unprotected) arms of an elevated plus maze and decreased defecation in a brightly lit chamber, both of which are consistent with a reduction in anxiety. The ventral HP lesioned animals also showed less of an increase in corticosterone in response to confinement in the brightly lit chamber.

Fear conditioning tasks offer a test of spatial (context fear) and non-spatial (cued fear) memory where performance is motivated by emotion. For the dorsal HP the data are clearer, dorsal lesions are known to cause an impairment in retention of contextual as opposed to cued fear (Kim and Fanselow, 1992).

On the contrary, ventral HP lesions or infusions of muscimol (which temporarily inactivates neurons) block tone fear and produce less consistent effects on context fear (Maren and Holt 2004). This role of the ventral HP in Pavlovian fear is consistent with the suggestions of the Moser group that the HP regulates emotion. A possible explanation is that ventral HP manipulations alter fear conditioning by depriving the amygdala of both dorsal and ventral HP information. The amygdala has a very general role in mediating fear memory and only receives direct HP input via the ventral HP (Maren and Fanselow 1995).

These results about a specific role of the DH in spatial memory were contradicted by other studies, (Ferbinteanu, Ray et al. 2003) using a "match-to-position" version of the water maze where deficits in spatial memory were produced by both dorsal HP and ventral HP lesions.

The reason of these discrepancies can be that is very difficult to anatomically define and distinguish the dorsal from the ventral HP, or separated anatomical subregions along the rostro-septal axis of the HP.

For this, gene expression data can help. A recent high-resolution genome-wide analysis revealed that pyramidal neurons in both CA1 and CA3 display clear regional and laminar specificities in C57Bl/6 mice. Using these robust gene markers, both of these fields were parceled into multiple, spatially distinct molecular domains and subdomains (Thompson CL and Pathak SD et al., 2008). Both CA1 and CA3 are divided respectively into three major molecular domains: dorsal (CA1d and CA3d), intermediate (CA1i and CA3i), and ventral (CA1v and CA3v) (Dong, Swanson et al. 2009) (Fig. 19 B). Finally, gene expression in the dentate gyrus also displays distinct regional specificity.

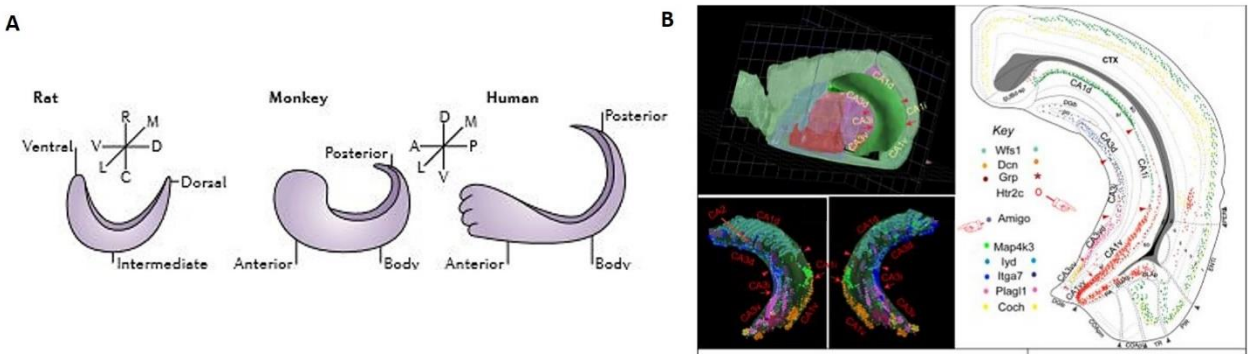


Fig. 19. *Schematic illustrations of the HP long axis in rats, monkeys and humans (Strange et al., 2014)* (**A**)**.** *Molecular domains of the HP CA1 and CA3. Representative marker genes expressed selectively in CA1, CA2 and CA3. Expression of these genes reveals a clear segregation between the dorsal and ventral areas* (**B**)**.**

A gradient expression along dorso-ventral axis of the HP was found for the nuclear receptor COUP TFI (chicken ovalbumin upstream promoter transcription factor 1, also called Nr2f1)

suggesting a role for this gene in regulating the differential growth and functional organization of the HP longitudinal axis. It is expressed in a low dorsal to high ventral gradient during HP postnatal development and the loss of function of this gene results in a progressive dysmorphic HP with altered shape, volume and connectivity, particularly in its dorsal and intermediate regions (Flore, Di Ruberto et al. 2017) (Fig.20).

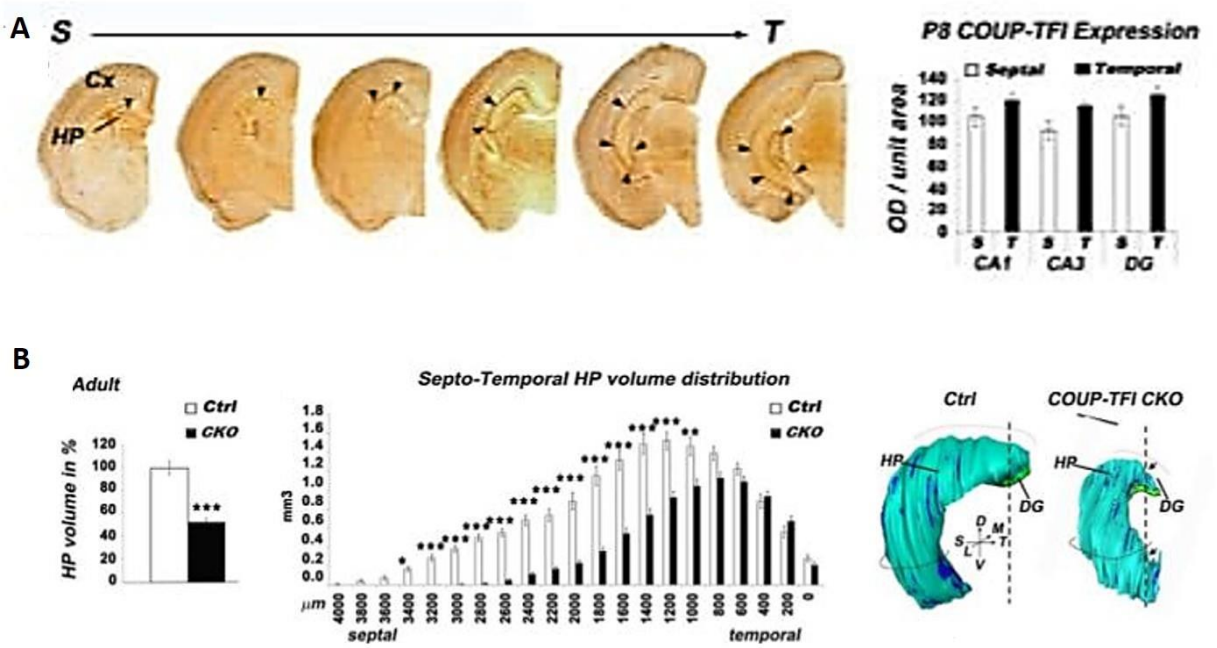


Fig. 20 *COUP TFI is expressed in a gradient fashion, which gradually increases dorso-ventrally along the anterior-posterior axis of all HP subfields* (**A).** *In COUP TFI conditional Knock-out mice in which COUP TFI is inactivated in all cortical and archicortical neuronal progenitors, there is an overall HP volume reduction of 47%. The three dimensional model representations of isolated control and mutant HP better illustrate the strong decrease of the dorsal portion (Flore et al., 2017)* (***B***).

These recent genetic data supported the segregation suggested by anatomical connection data and behavioral results and more clearly demarks these regions.

Hippocampal pathologies: a dorso-ventral classification

The HP has been implicated in several neuropsychiatric disorders. Environmental factors play a large role in determining HP integrity more than genetic factors. The advent of MRI in the last few decades has witnessed an escalation of HP volumetric studies in various neuropsychiatric disorders. The medial temporal limbic area is specifically affected in Alzheimer's disease (AD) and temporal lobe epilepsy (TLE). Similarly, the psychotic features of schizophrenia have been attributed to abnormal HP activity and a disturbance of HP–cortical connections. Volumetric alterations of the HP have been found in stress and anxiety-like disorders such as depression and posttraumatic stress disorder

(PTSD). In temporal lobe epilepsy, hippcampal sclerosis and HP atrophy have been found. In AD HP volume loss is a manifested morphological abnormality of the disease. HP volume declines with age, and HP volume loss is generally present in demented patients, and in mild cognitive impairment. Traumatic brain injury is also associated with bilateral HP volume loss. In mild cognitive impairment, the HP volume loss has been shown to be an early marker for developing AD later. HP volume reductions have been found in schizophrenia early in the course of the disease. In rats, neonatal excitotoxic lesions disrupt development of the prefrontal cortex and transient inactivation of the ventral HP during a critical period of development may also produce subtle anatomical changes in the HP, sufficient to disrupt normal maturation of the prefrontal cortex (Lipska, Halim et al. 2002). In Parkinson's disease it has been proposed that demise of the entorhinal cortex in PD (through the presence of neurofibrillary tangles) isolates the HP from its isocortical inputs and thus causes volume loss (Laakso, Partanen et al. 1996). Moreover, HP lesions were caused by α-synuclein-positive Lewy bodies abundant in the CA2/3 region in all Parkinson's disease (PD) and Dementia Lewy bodies (DLB) cases, in addition to α-synuclein-positive Lewy bodies in entorhinal cortex. In addition, many hilar neurons in both PD and DLB cases were surrounded by accumulations of α-synuclein-positive punctate or vesicular profiles. This suggests the existence of aggregates of α-synuclein in axon terminals of dentate mossy fiber projections to hilar neurons. Axonal spheroid-like lesions were also identified in the stratum moleculare of the dentate gyrus of PD and DLB cases with antibodies to γ-Synuclein (Galvin JE, Uryu K et al., 1999). The Synucleins are a family of soluble presynaptic proteins that are abundant in neurons and include α-synuclein, β-synuclein and γ-synuclein. Their function is poorly understood but, probably they may play a role in synaptic transmission. α-Synuclein become less soluble in neurodegenerative disease, which may lead to the formation of fibrillar aggregates in the form of LBs. Because the α-Synuclein-positive lesions appear to be predominantly localized to abnormal aggregates in the mossy fiber terminals that synapse on hilar neurons, and the neuritic pathology labeled by antibodies to α-Synuclein is most likely in axon terminals of CA2/3, these abnormal processes may impair synaptic transmission in HP perforant pathway projections critical to memory and behaviour. The aggregation of α-Synuclein labeled vesicles and filaments in presynaptic terminals might reflect impaired synaptic vesicle release and synaptic dysfunction. Moreover, the observation that the pathology in the hilus and dentate gyrus also contains the synaptic proteins synapsin, synaptophysin, and synaptobrevin suggests that these lesions are causing degeneration in presynaptic terminals. The accumulation of α-Synuclein proteins in the degenerating terminals described here could interfere with the unidirectional flow of information in the HP, which is an important component of the neuroanatomical circuits involved in memory and behaviour. Therefore, α-synuclein-positive Lewy bodies present in the HP of Parkinson's disease

patients and patients with DLB are responsible of the memory deficits in these disease. How α-synuclein overexpression in dorsal HP affects memory I investigated in study 2.

Moreover, anterior–posterior differences in the relative severity of HP structural and functional changes in various psychiatric and neurological conditions are emerged. For a number of these conditions, preclinical animal models have considerable predictive value regarding the relative severity of anterior versus posterior pathology observed in patients (Fig. 21). In addition, the locus of pathology on the long axis is associated with specific cognitive impairments (for example, schizophrenia is associated with anterior hippcampal pathology and with impaired transitive inference (Ongur, D., Cullen TJ et al., 2006). For example, in view of the greater connectivity between the ventral (anterior) HP and endocrine hypothalamic nuclei impaired hormonal regulation by the hypothalamus (such as hyponataraemic polydypsia reported in patients with schizophrenia who have decreased anterior HP volume (Goldman, Marlow-O'Connor et al. 2008) may be a common finding in patients with anterior HP damage. Furthermore, given the role of the anterior HP in models of innate anxiety, this region could prove to be an important future target for a range of neurotic disorders.

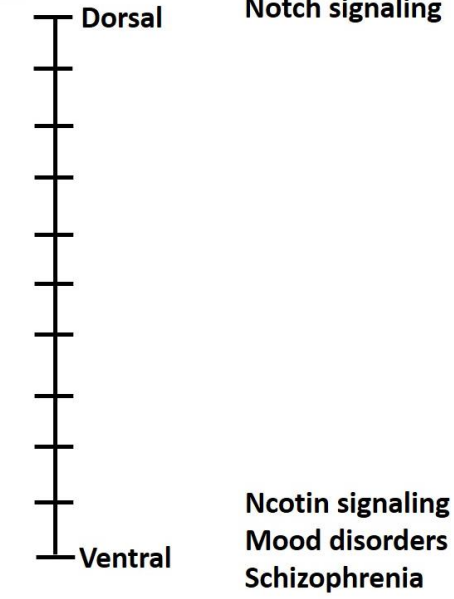
Condition	Abnormality along hippocampal long axis	
	Animal	Human
Medial temporal lobe epilepsy	Greater spontaneous epileptiform bursting in the ventral hippocampus than in the dorsal hippocampus[15,184]	• Chronic intracranial recordings in patients indicate that seizure initiation is more frequent in the anterior hippocampus than in the posterior hippocampus[185] • Neuronal loss is greater in the anterior hippocampus than in the posterior hippocampus[186–188] (expressed as an anterior–posterior gradient[186])
Depression	Behavioural effects of chronic antidepressant treatment are critically dependent on adult neurogenesis in the hippocampus[189], and this has been suggested to occur specifically in the ventral hippocampus[190]	Post-mortem studies on patients with major depressive disorder show that antidepressants increase neurogenesis in the anterior dentate gyrus[191]
Schizophrenia	• Lesioning of the ventral hippocampus is used to model several features of schizophrenia[192] • Schizophrenia-related biomarkers are present in the ventral hippocampus at birth[44]	Increasingly thought that the primary pathology is in the anterior hippocampus[160], but there is also considerable evidence for abnormalities in the posterior hippocampus (for example, see REFS 193–194)
Ischaemia	• Ventral-to-dorsal increase in hippocampal vulnerability to ischaemia[195] • May be related to an increasing gradient for NMDA receptor expression from ventral to dorsal in area CA1 (REF. 196), as NMDA receptor activation has been proposed to have a role in hypoxic excitotoxicity[197] • Cerebral blood flow is greater in the ventral hippocampus than in the dorsal hippocampus during reperfusion following ischaemia, which may contribute to dorsal hippocampus damage[198]	Posterior hippocampus volume is decreased in patients who have had cardiac arrest with successful subsequent resuscitation[199] (but note previous reports of cardiac arrest-induced ischaemia affecting the entire hippocampal long axis[200])

Fig. 21 Preclinical animal studies provide insights into the locus of HP damage in different patient populations (Strange, Witter et al. 2014).

A recent challenge has been to investigate whether the genetic composition of these subdomains can be related to specific pathologies. Of the ten molecular pathways enriched in the dorsal HP at all ages, three were related to Notch signaling and two were cell adhesion pathways. Notch1 has also been

shown to have a role in adult HP long-term potentiation and spatial learning and memory (Costa RM, Honjo T. et al. 2003). The ventral pole was found to predominantly have enrichment of nicotinic signaling pathways over the dorsal pole. A lot of these genes are "biomarker" that have been reported in literature to be associated with diseases. The disease-related biomarkers found to be enriched in the dorsal HP were mostly related to cancers and inflammation. In contrast, in the ventral pole, biomarkers for diseases revealed relevant central nervous system diseases. Specifically, enriched in the ventral portion of the HP were biomarkers for schizophrenia, affective disorders, and addiction (Fig. 22). Such differentiation in gene expression along the dorsal–ventral axis is present from the birth suggesting that the dorsal–ventral axis of HP at birth shows adult-like functional differentiation. In fact, the neonatal ventral HP is enriched with biomarkers associated with mental illnesses suggesting an early developmental susceptibility of the ventral HP to mental illness (O' Reilly KC and Flatberg A et al., 2014).

A



B

Dorsally enriched diseases	Ventrally enriched diseases
Connective tissue diseases	Affective disorders, psychotic/bipolar disorder
Aortic aneurysm, thoracic	Mental disorders/psychiatry and psychology
Stomach neoplasms/ stomach diseases	Schizophrenia/schizophrenia and disorders with psychotic features
Rheumatic diseases	
Musculoskeletal diseases	Anxiety disorders
Eye diseases	Mood disorders
Ameloblastoma	Alcoholism/alcohol-related disorders
Arthritis, rheumatoid	
Neoplasms, neuroepithelial	

Fig. 22 *Indicated are the main signaling pathways enriched in dorsal or ventral HP* **(A).** *Diseases enriched in dorsal and ventral HP at all ages (based on biomarkers) (O'Reilly et al., 2014)* **(B).**

Study 1. Functional differences between dorsal and ventral hippocampus in object and spatial working memory capacity

Aim

The normal human brain never reaches a point at which new experiences can no longer be committed to memory. At the same time, one can be overwhelmed by new information to the point that it seems to be too much to retain in memory. The feeling of being overwhelmed by new information can occur because of the special type of memory that is typically termed *working memory* (WM). It refers to the relatively small amount of information that one can hold in mind, attend to or remember in a rapidly accessible state. The term *working* is meant to indicate that mental work requires the use of such information. The amount of information that can be held in mind successfully at one time is defined as *working memory capacity* (WMC). The concept of WM and its limits is a key part of the human condition. We need WM for solving problems, for language comprehension, for arithmetic, to remember partial results until the rest of the answer can be calculated, for reasoning and for most other types of cognitive tasks.

The question subject of debate has been to define what is the human limit in WM. According to a very broad definition, WMC is simply the ability to remember things in an immediate-memory task (a task with no delay between the end of the presentation of items to be recalled and the period of recall itself). For example, if given a list of words to recall, one recalls six words, one's capacity would be six words; if one recalls three words, one's capacity would be three words and so on (Cowan, Rouder et al. 2012). Therefore, one of the major limitations in human cognition is the limited amount of information that can be processed by WM (Cowan 2001). Individual differences in WMC are associated with variation in several important abilities, including control of attention, non-verbal reasoning abilities (Kyllonen P.C and Christa R.E. et al., 1990) and academic performance (Gathercole, S. E. et al. 2003).

Reduced WMC is also a feature of many clinical populations, including individuals with schizophrenia, stroke, traumatic brain injury and attention deficit- hyperactivity disorder (ADHD). WMC is decreased in Parkinson' s disease, as shown by impaired short-term recall in tasks requiring the inhibition of interfering stimuli(Wilson, Kaszniak et al. 1980), digit ordering (Cooper, Sagar et al. 1992) and spatial organization (Bradley, Welch et al. 1989). Parkinson's disease patients are able to hold fewer items in memory than control subjects (Lee, Cowan et al. 2010). Moreover, patients with Alzheimer's disease also show a reduced span (Wilson, Bacon et al. 1983).

The neural basis regulating WM and its limits is still debated. Elucidating this is a key goal in cognitive neuroscience. Lesion studies first associated WM with the prefrontal cortex (Pribram, Mishkin et al. 1952) and this link was later confirmed by electrophysiological recordings that revealed neural correlated of WM in the PFC of monkeys (Kubota and Niki 1971). In addition to neurophysiological studies in non-human primates, much research on the neural basis of WM has been carried out in rodents. One of the tasks that is suggested to be a rodent equivalent to human and macaque WM tasks is the eight-arm radial maze, in which information about the location visited needs to be remembered across consecutive choices. Lesions of the prelimbic region, a part of the cortex that is suggested to be a homologue of the primate prefrontal cortex (PFC), impair performance on WM tasks in rodents (Delatour and Gisquet-Verrier 2000).

Recent evidence reports a role for the HP in regulating WM, in contrast with the theory of a selective role for the HP in long- term memory (Broadbent, Squire et al. 2004). Olton (Olton and Papas 1979) demonstrated that lesions of HP structures in rats also produce an impairment in WM, as measured by performance on the radial arm maze. Much more controversial has been the role of HP in item WM. Nevertheless, recent studies demonstrated that item WM is affected in patients or monkeys with HP damage and in amnesic patients in conditions of high information load (Beason-Held, Rosene et al. 1999, Levy, Manns et al. 2003). Studies in rodents about the involvement of the HP in item WM are few and quite recent. Large HP lesions in rats did not affect odor span but impaired the performance in a spatial span task (Dudchenko, Wood et al. 2000). A recent study reported that CD1 mice have an object memory span of about six different objects and lesions in the dorsal HP affected object WM in high memory load conditions (Sannino, Russo et al. 2012), thus supporting previous results obtained in primates.

The aim of this study was the elucidation of the role of HP in mediating spatial information-related WMC and objects-related WMC. In order to address this issue, we are going to take into account recent evidence suggesting that the HP is not a unitary structure, but as a segregated structure in a dorsal (septal pole) and a ventral (temporal pole) portions taking on different cognitive and behavioural functions (Moser and Moser 1998). The dorsal HP performs primarily cognitive functions while the ventral HP relates to stress, emotion and affect (Fanselow and Dong 2010). Several genes are differentially enriched in the adult dorsal and ventral HP as in the neonatal dorsal and ventral HP (O'Reilly, Flatberg et al. 2015, Flore, Di Ruberto et al. 2017). A lot of these differentially enriched genes are biomarkers for diseases. The disease-related biomarkers enriched in the dorsal HP were mostly related to cancer and inflammation (PGE2 pathways, Cadherin 8 (Cdh8) (Flore, Di Ruberto et al. 2017). In the ventral HP were found biomarkers for relevant central nervous

system diseases as schizophrenia, affective disorders and addiction (Nicotine signalling, Neuronal growth regulator 1 (Ngr1), LIM domain transcription factor Lmo4, COUPTII (Flore, Di Ruberto et al. 2017). These genetic differences, together with known anatomical differences (Amaral and Witter, 1995) demonstrated the heterogeneity of the HP.

Since the HP is not a functionally homogenous structure, in this study we asked whether there is a functional difference between dorsal and ventral HP in regulating WMC. Given that WMC deficits are found in many diseases, elucidating which one mostly regulates object or spatial WMC is useful to have a common target for them.

To this aim we performed selective excitotoxic lesions in dorsal and ventral HP in CD1 mice and then tested these mice in the 6 DOT/ 6 IOT and in a WMC version of the radial arm maze task in order to investigate the role of these two regions in objects and spatial WMC.

Materials and methods

Subjects

The subjects were outbred male CD1 adult (10-16 weeks) mice (Charles River, Italy, RRID: rid_000091), weighting 40-50 g before stereotaxic surgery. They were housed in groups of 3-5 mice *per* cage. The housing condition was maintained at 24±1 °C and 55±5% relative humidity, with a 12-h light: 12-h dark cycle (lights on 0730- 1930). Mice were tested during the light phase. The experiments were conducted in accordance with the European Communities Council directives and Italian laws on animal care.

Surgery

Mice were randomly assigned to groups that received either bilateral excitotoxic lesions of the dorsal HP (dorsal lesion) or ventral HP (ventral lesion) or were vehicle-injected mice (control).

The surgical operation was performed according to the method described in Sannino et al. (Sannino, Russo et al. 2012). Mice were anesthetized by an intraperitoneal (i.p.) injection of Avertin (2,2,2-Tribomoethanol 97%, Sigma Aldrich SRL, Milano, Italy) (20 µl per gram of mouse body weight) and then fixed on a stereotaxic apparatus (David Kopf Instruments). An injection needle was bilaterally inserted within the HP. The stereotaxic coordinates for the bilateral dorsal HP lesion were AP = - 1.9 mm, L= ± 1.2 mm, D= - 1.6 mm from bregma, for the ventral HP lesion were AP= -4.1 mm; L=±3.0 and DV=- 3.7 mm from bregma according to the mouse brain atlas (Franklin and Paxinos 2001). The needle (0.20/0.09 x 30 mm; Unimed Svizzera) was connected by plastic tubing (1.09/0.38 mm, 2Biological Instruments, Varese, Italia) to a 2.5 µL Hamilton syringe, which allowed the injection of 0.3 µl/side of 20 mg/mL N-methyl-D-aspartate (NMDA; Sigma-Aldrich, St. Louis, MN) dissolved in 1% phosphate-buffered saline (PBS, pH 7.4). The control group of animals was injected with only 1% PBS. Behavioral testing started 10–15 days after surgery.

Histology

Following testing, all animals were intracardially perfused, under Avertin anesthesia, with PBS (pH 7.4) and then with the buffer containing 4% paraformaldehyde. Brains were removed and stored in

4% paraformaldehyde for a week prior to being sectioned on a vibratome. They were sectioned at 50 µm and every second section was mounted and stained using the Nissl method. The slices were acquired using a Leica stereomicroscope (1X objective) and then analysed.

Analysis of Lesions

Lesioned areas were defined as the areas of cellular loss evident with the Nissl method. Only animals with correct placements of the lesion were included in statistical analysis. In order to ensure similarity of the lesion, a set of criteria for histological examination prior to including the animals in final data analyses were used.

Dorsal HP was defined as the HP region extended between the coordinate -0.94 mm and the coordinate -2.30 mm from the bregma. Ventral HP was defined as the HP region extended between the coordinate -2.46 mm and the coordinate -3.80 mm from the bregma. The criteria used were: 1) the lesion had to be only confined to the HP and not to others extra-HP structures, 2) no lesion or only a lesion in more posterior CA3 area of the dorsal HP for the ventral lesion group.

Experimental design

Animals were tested in different behavioural tasks with the following order: plus maze task (day 1), 6 Different objects recognition task (6 DOT) (day 2), 6 Identical objects task (day 10), radial arm maze task (confinement and no-confinement procedure or no-confinement procedure, not preceded by a confinement procedure (day 15- day 32)). In the no-confinement procedure, not preceded by confinement procedure, the animals were exposed to the same tasks except for the radial arm maze task where only the four days of the no-confinement procedure, not preceded by confinement procedure were performed (see below) (Fig. 23).

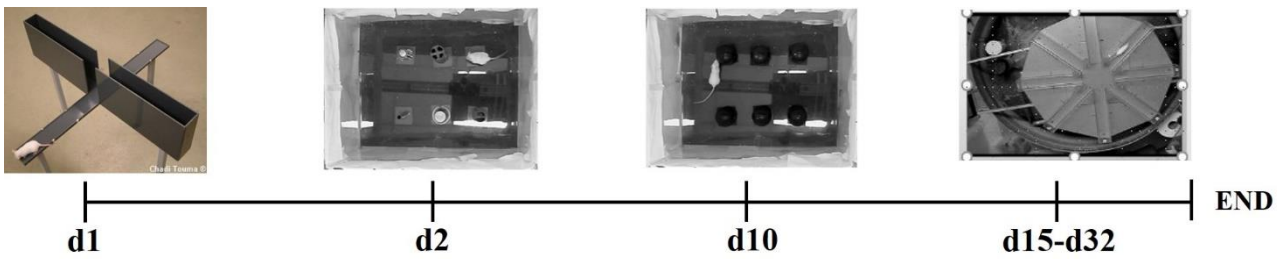


Fig. 23 Experimental design. *Control, dorsal HP and ventral HP lesion groups were exposed to the plus maze task (day 1, d1), to the 6 DOT (day 2, d2), to the 6 IOT (day 10, d10) and to the radial arm maze task (day 15-day 32, d15-d32).*

Behavioral procedures

Elevated plus maze

In order to verify whether our lesions damaged the same areas described in literature, we first tested our mice in the elevated plus maze task, a task classically associated to the function of the ventral HP (Bannerman, Grubb et al. 2003). The elevated plus maze is widely used in literature to test anxiety and to validate new anxiolytic drugs because it has high predictive and construct validity. It evaluates an animal's relative tendency to leave the safety of walled enclosures (the closed arms) for more open areas (the open arms). The apparatus consisted of two opposing open arms (37 x 9cm) and two closed arms (37 x 9cm) that extend from a common central platform (8 x 8 cm). The maze was elevated from the floor 50 cm. The apparatus was located in a dimly lit room (100 W light). For each test, individual animals were placed in the center of the maze, facing an open arm and they were allowed to move freely for 5 min. The test was videotracked by a camera mounted on the ceiling and connected to a videotracking system (AnyMaze, Stoelting, USA). The percentage of time spent in open arms and the percentage of open arms entries were measured. More time and more entries in the open arms were considered an index of less anxiety.

6 DOT/6 IOT

6 DOT and 6 IOT were performed as described in Sannino et al. (Sannino, Russo et al. 2012). The 6 DOT allows to measure the WM in high memory load conditions corresponding to a high number of objects to remember in a short-time interval. In the 6 DOT mice were isolated for 15 minutes in a waiting cage before testing and then subjected to a habituation period of 10 minutes in an empty arena (35 x 47 x 60 cm), T1 phase. Habituation period allows assessing motor impairment. After 1 min spent in their waiting cage, they were subjected to the study phase (T2 phase), during which they were allowed to explore 6 different objects for 10 min or for a maximum of 120 sec of total objects exploration. Exploration was defined as the time in which the nose was in contact (< 2 cm from the object) with the object. After 1 min of intertrial interval (ITI), animals were subjected to the test phase, T3 phase during which the objects are replaced with identical copies of the familiar objects and a new object. Two different new objects were used and the position of new object was changed

across animals in a random order to avoid any bias linked to the object used or to the position. Animals were allowed to explore the objects for 5 min. Animal's behaviour was recorded for 5 min by a video-tracking system (Any-maze, Stoelting, USA) and analyzed by a trained observer. In the 6 IOT animals were exposed to identical copies of the same objects. This allowed to increase the number of objects without increasing the memory load, and therefore to use it as control task for the 6 DOT. In this case, T1 phase was identical to T1 phase in the 6 DOT. In T2 phase, mice were allowed to explore objects for 5 min or for a maximum of 35 sec of total objects exploration. In the T3 phase, the objects were replaced with identical copies of the familiar objects and a new object. Animals were allowed to explore the objects for 5 min. Animals were exposed to 6 DOT and to 6 IOT 8 days apart.

Exploration of each object was measured. New object discrimination was considered to occur when the new object was explored significantly more than all the other familiar objects, tested with Duncan *post-hoc* test.

Eight arms radial arm maze

Confinement and no-confinement procedure

In order to evaluate the role of dorsal and ventral HP in spatial WMC we performed a WMC version of the radial arm maze task first using a confinement procedure to avoid animals to use egocentric spatial memory to solve the task, then using a no-confinement procedure where they are free to use allocentric or egocentric spatial memory. The apparatus was a home-made wooden radial arm maze with a painted grey flooring. It was elevated 84 cm above the floor and consisted of eight identical and equally spaced arms (38 cm long x 8 cm wide x 9 cm high) radiating from a central octagonal platform (19 cm in diameter). Doors made of transparent Plexiglas (9 cm high) were placed if necessary at the entrance of each arm. The maze was located in a well-lit room and it was enclosed by grey curtains. Four visual extramaze cues were located around the maze. A confinement box, hand-made with four transparent rectangular Plexiglas pieces glued together and open on one side to allow the animal to enter, was used in the confinement phase. It was a removable box put by the experimenter at the exit of an arm every time the animal went in an it. In this way, the experimenter waited for the animal and blocked it inside the box when it went out the arm. It was a homemade system to confine the animal between each arm choice and avoid that it developed egocentric strategies (otherwise developed when it is free to explore the apparatus) to solve the task. The experimenter was in the same fixed position for all the duration of the test. A small piece of cocoa

cereals (Coco Pops, Kellogg's) was placed at the distal extremity of each arm and used as reward. The day before the beginning of the test and for all the duration of the test, mice were maintained on a restricted feeding schedule designed to keep their body weight at 80-85% of the free-feeding level, while the water was available *ad libitum*.

The confinement and no-confinement procedure consisted of a habituation phase, a pretraining (PT) phase, a WMC training phase with the confinement (first 4 days) and a WMC training phase with the no-confinement (next 4 days). During the weekends, the animals were not tested but they were maintained on the restricted feeding schedule (Fig. 24). The test started with the habituation phase (3 days, 1 trial per day, 10 min per trial) to habituate the animal to the apparatus, to the food reward and to the confinement box. During the habituation phase, all arms were open and twenty pieces of cocoa cereals were placed in the apparatus (two for each arm, one at the entrance and one at the end of the arm and four in the central zone). On the second day of habituation, the confinement box was introduced to habituate them to it. Every time the animal went out an arm, the confinement box was placed at the proximal extremity of the arm and, if the animal did not spontaneously enter it, it was gently accompanied in it.

After the habituation phase, the PT phase took place to train the animals to the mechanism of the task. It consisted of 9 training trials per day for 2 days with only two open and baited arms; the others 6 arms were closed. The open and baited arms were different between trials within the same day, and also within trials between days.

At the start of each trial, the animal was placed in the center of the apparatus. Every time the animal entered an arm, the experimenter placed the confinement box at the proximal end of the arm and when the mouse was in the box, closed it inside the box using the Plexiglas door used to close the arm. The animal was confined for 5 seconds after which it was free to move on the apparatus. During the trial, the experimenter recorded the sequence of arms entries made by the animal and the time when the animal entered the last open and baited arm. An arm entry was considered when the animal puts the four paws beyond the middle of the arm. The trial ended when the animal had visited the two different arms or 6 min had elapsed. After the PT phase, a WMC training phase with the confinement took place.

In the WMC training phase, WM load was modulated by modifying the number of open and baited arms. It consisted of 9 training trials per day for 4 consecutive days in which the number of open and baited arms changed from 3, 6 and 8 open and baited arms: 3 trials with 3 open and baited arms (the other 5 closed), 3 trials with 6 open and baited arms (the other 2 closed), and 3 trials with 8 open and baited arms. The order of the trials and the choice of the open and baited arms were random within

day and between days. A trial ended when an animal entered all the open and baited arms or when 6 minutes had passed. The sequence of arms entries of the animal and the time to complete the trial (when the animal entered all open and baited arms) were recorded.

After two free days where the animals were not tested but they were maintained on the restricted feeding schedule, described previously, four days with no – confinement (without confinement box) took place. It consisted of 9 training trials per day for 4 consecutive days in which the number of open and baited arms changed from 3, 6 and 8 open and baited arms as in the WMC training phase with the confinement. The trial ended when the animal entered all open and baited arms or 6 minutes had elapsed. The measure analysed in both PT phase and WMC training phase (confinement and no-confinement procedure) were the mean number of errors with 3, 6 and 8 open and baited arms. Errors (expressed as the total number of entries (recorded by the experimenter during each trial) – correct entries (entry only once in an open and baited arm) were defined as re-entering in open arms already visited previously. Time to complete the task and mean score of the egocentric strategies made in the trials with 3, 6 and 8 open and baited arms (sequential and alternating strategies, described in detail below) were also analysed in the WMC training phase.

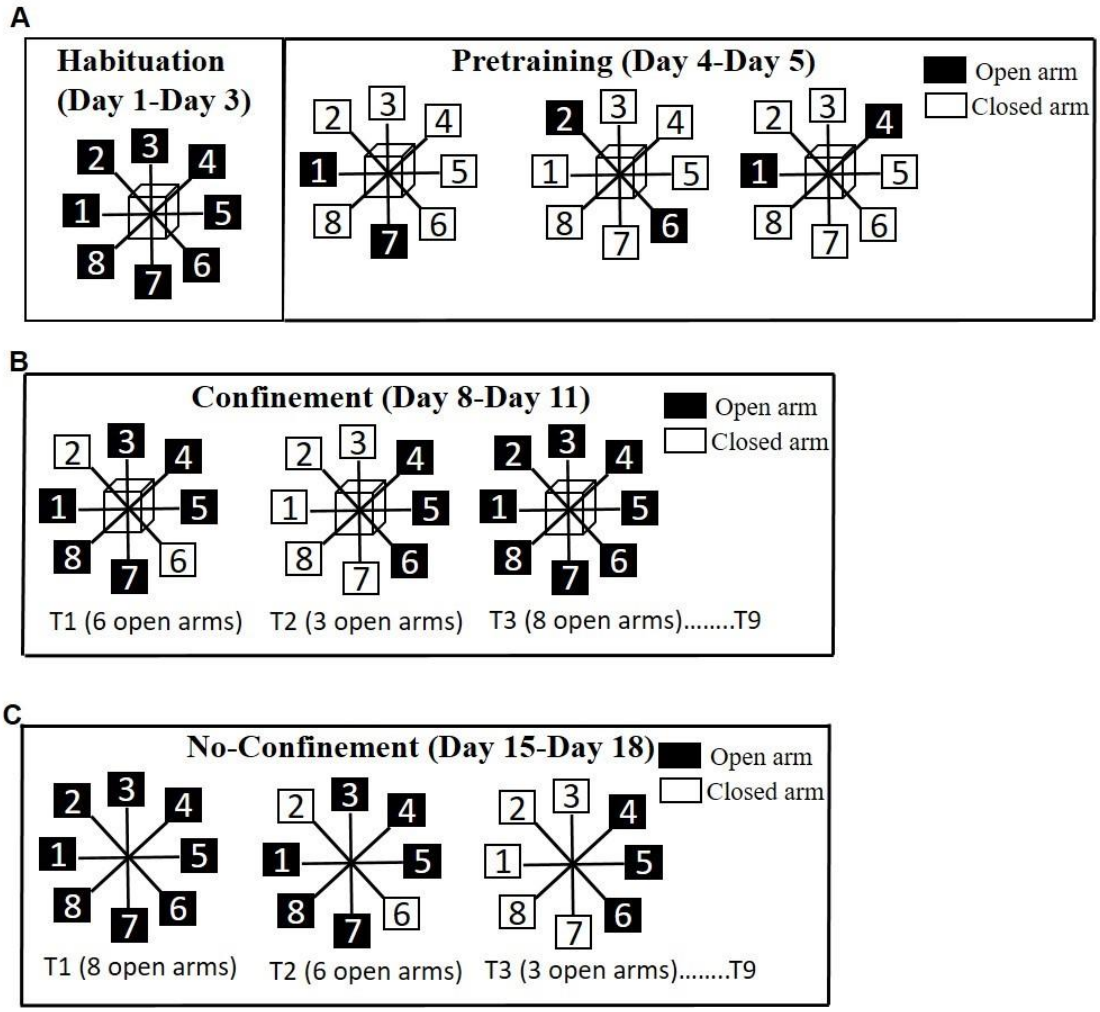


Fig. 24 WMC radial arm maze task (confinement and no-confinement procedure). *Habituation phase (day 1-day 3) and PT phase (day 4- day 5) (**A**), confinement procedure (day 8-day 11) (**B**) and no-confinement procedure (day 15- day 18) (**C**).*

No- confinement procedure, not preceded by a confinement procedure

Another control group, another dorsal HP lesion group and another ventral HP lesion group were tested in a no-confinement procedure, not preceded by a confinement procedure in order to evaluate performance in the no-confinement procedure, not influenced by a previous exposure to the confinement procedure. In the no-confinement procedure, not preceded by a confinement procedure of the radial arm maze task, the apparatus and the experimental room were the same used in the confinement and no-confinement procedure. In this case animals were only exposed to the procedure without the confinement.

The day before the test and for all the duration of the test, animals were maintained on the same restricted feeding schedule described previously.

The test consisted of a habituation phase, a PT phase and a WMC training phase with no-confinement. During the habituation phase animals were allowed to explore the apparatus for a 10-min trial each day for 3 days. In this phase 20 pieces of cocoa cereals (food reward) were scattered throughout the maze. In this phase animals were habituated to the apparatus and to the food reward. The confinement box was not used.

The PT phase consisted of 9 training trials per day for 2 days with only two open and baited arms. The open and baited arms changed within the trials and between the two days. The trial ended when the animal has visited the two different arms or 6 min had elapsed.

The WMC training phase consisted of 9 training trials per day for 4 days. The procedure was the same described in the confinement and no-confinement procedure. The trials changed from 3, 6 and 8 open and baited arms and the order of the trials was random within day and between days. The trial ended when the animal had visited all open and baited arms or 6 min have elapsed.

The measures taken in account were the mean number of errors and the score of the sequential and the alternating strategies.

A database for measuring behavioural strategies in the radial arm maze task

A radial arm maze (RAM) database was made in collaboration with the bioinformatics core of the Telethon Institute of Genetics and Medicine (TIGEM) to analyse data scored from the radial arm maze task. The webtool RAM analysis was a collection of scores automatically obtained from manually inserted data. It was implemented with HTML, PHP and Javascript languages. The loaded and analyzed data were stored in a PostgreSQL database. It was organized in different sections: 1) ***Home page,*** an overview of the radial arm maze task and relates videos, 2) ***Animal page,*** where you could insert the new animal and the relative information (Birth date, place of birth, age, sex, treatment, lesion type) and edit it at any time, 3) ***Training page,*** where you reported for each inserted animal the arms entries, the time and the body weight for all trials in every training day, 4) ***Archive page,*** where you found the automatically calculated scores regarding the strategies used in the radial arm maze, the span and the perseverative behavior (Fig. 25).

The sequential strategy measured in the analysis was defined as the longest sequence of adjacent arms made by the animal in a trial and was calculated by adding +1 for each arm of the sequence. The strategy was calculated taking in account whether in the sequence there was an arm re-entry (-1 for each arm re-entry) or not taking in account it. The first was defined "Sequential minus error (SeqMErr)". For example, if the sequence of entries was 1-**2-3-4-5-6-7-8**, the score of the sequential strategy was 8 (1 point for each arm in bold). If we considered that in the sequence 3-2-**3**-4-5-6-7-8, 3 (in bold) was a re-entry, we had to subtract 1 in the score of the SeqMErr (measure not shown in the results). The final score was 7.

The alternating strategy was also measured by adding +1 every time the animal entered in an alternating arm. For example, in the sequence 1-3-5-7, the score was 3.

Other measures were analysed. The perseverative behaviour calculated as the number of times that an animal re-enters consecutively in the same arm (measure not shown in the results).

For example, in a sequence 1-2-3-**3-3**, the score of the perseverative behaviour was 2 (2 re-entries in the arm 3, in bold). Finally, the span was calculated as the number of arms entries before committing an error. A span Minus Sequential was also measured subtracting the score of the sequential strategy made in the first entries before to commit an error (measure not shown in the results). All these measures were automatically calculated by the software after the experimenter had entered the data recorded during the task.

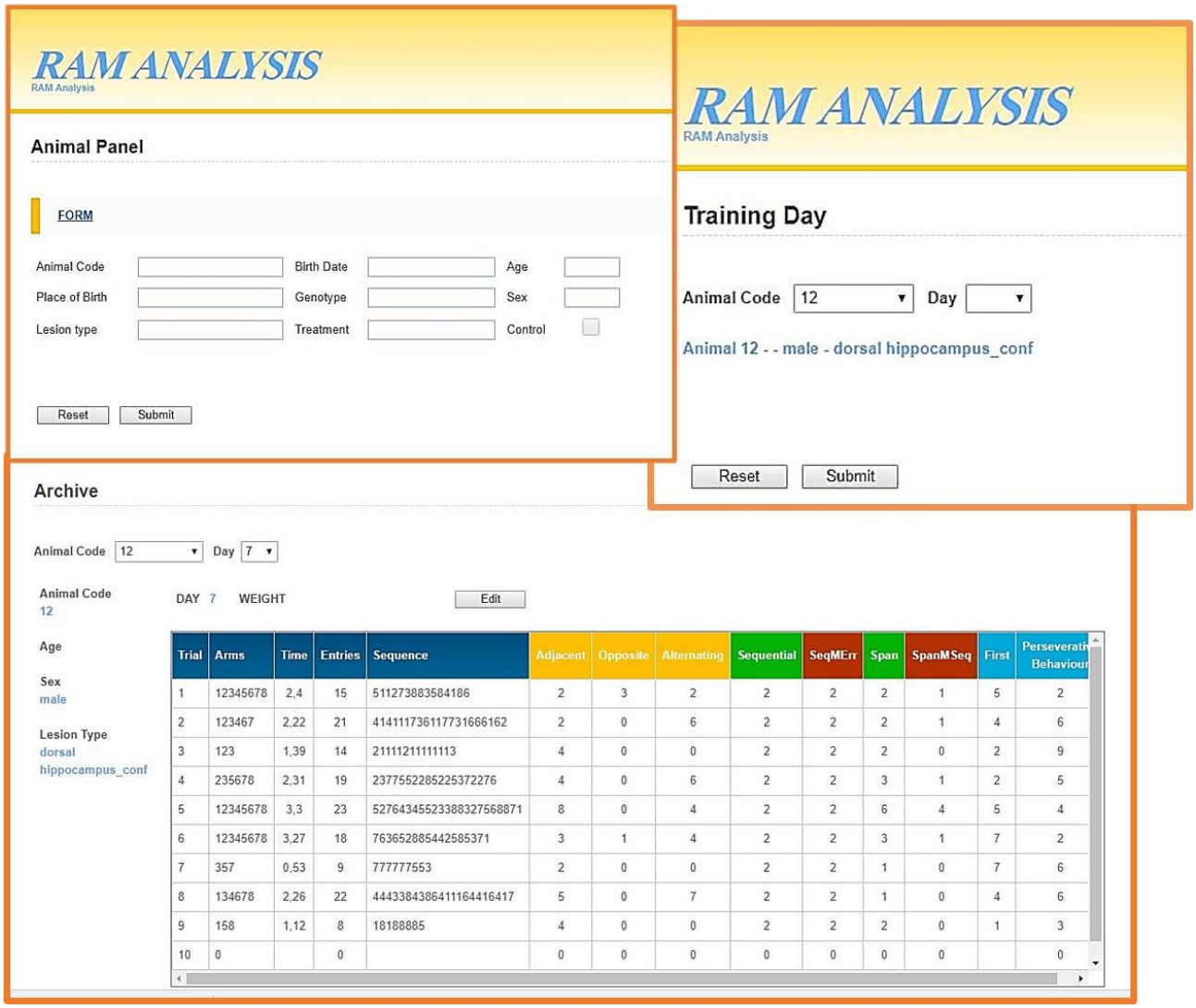


Fig. 25 Schematic representation of the different sections in RAM analysis database. *From left, Animal panel to insert the characteristics of the animal, training day to insert arms entries, time to complete the task and the body weight and archive where the score of the different measures is automatically calculated.*

Statistical analysis

All data are expressed as mean ± SEM. An ANOVA analysis followed by a Duncan *post-hoc* analysis, when necessary, were performed.

Elevated plus maze: a one-way ANOVA with the treatment factor (3 levels: control (n = 11), dorsal HP lesion group (n = 8) and ventral HP lesion group (n =16) as between variable was used to analyse the percentage of open arms entries and the percentage of open arms time. Control group included both PBS-injected mice in the dorsal HP and PBS-injected mice in the ventral HP because differences in the performance in all task were not found.

6 DOT/6 IOT: a two-way ANOVA for repeated measures was used to test the effects of the treatment (between variable, 3 levels: control (n = 8), dorsal HP lesion group (n = 6) and ventral HP lesion group (n = 12)) on the objects exploration (repeated measures, 6 levels: New, F1, F2, F3, F4 and F5) in the 6 DOT and in the 6 IOT. The distance travelled in the T1 phase and the total object exploration in T2 phase in both 6 IOT and 6 DOT were analysed with a one-way ANOVA using the treatment factor as between variable.

Radial arm maze (confinement and no-confinement procedure): a one-way ANOVA with the treatment factor (3 levels: control (n = 10), dorsal HP lesion group (n = 8) and ventral HP lesion group (n = 12)) as between variable was used to measure mean number of errors in the PT phase.

A three-way ANOVA for repeated measures with the treatment factor (3 levels: control (n = 10, confinement procedure, n = 8, no-confinement procedure), dorsal HP lesion group (n = 8) and ventral HP lesion group (n =12)) as between variable and the number of open arms factor (3 levels: 3, 6 and 8) and the procedure (2 levels: confinement and no-confinement) as repeated measures was performed to analyse mean number of errors, the score of the sequential strategy, the score of the alternating strategy and the time to complete the task. A two-way ANOVA for repeated measure with the treatment factor (3 levels: control, dorsal HP and ventral HP lesion groups) as between variable and the number of open arms factor as repeated measures was performed separately on the confinement and on the no-confinement procedure to analyse the same measures.

A two-way ANOVA for repeated measures with the treatment factor (3 levels: control (n = 10, confinement procedure, n = 8, no-confinement procedure), dorsal HP lesion group (n = 8) and ventral HP lesion group (n =12)) and the day factor (5 levels: last day of confinement, day 1, day 2, day 3 and day 4 of no-confinement procedure) was performed to analyse the use of the sequential strategy in the no-confinement procedure across days.

Radial arm maze (no confinement procedure, not preceded by a confinement procedure): a two-way ANOVA for repeated measures with the treatment factor (3 levels: control (n = 13), dorsal HP lesion group (n = 10) and ventral HP lesion group (n = 9)) and the number of open arms factor (3 levels: 3, 6 and 8 open arms) as repeated measures was performed to analyse mean number of errors, the score of the sequential and the alternating strategy.

Statistical significance was set at $p \leq 0.05$. A Duncan *post-hoc* analysis was done when ANOVA analysis was found significant.

Results

Histological evaluation of the dorsal and ventral hippocampal lesion

Nissl staining on alternate coronal sections of the brain of lesioned animals, as compared to control animals, showed a wide cellular loss, with necrosis areas and alteration of the tissue. Photomicrographs of coronal brain sections from representative mice in the dorsal HP and ventral HP lesion groups as compared to respective controls are shown in Fig 26.

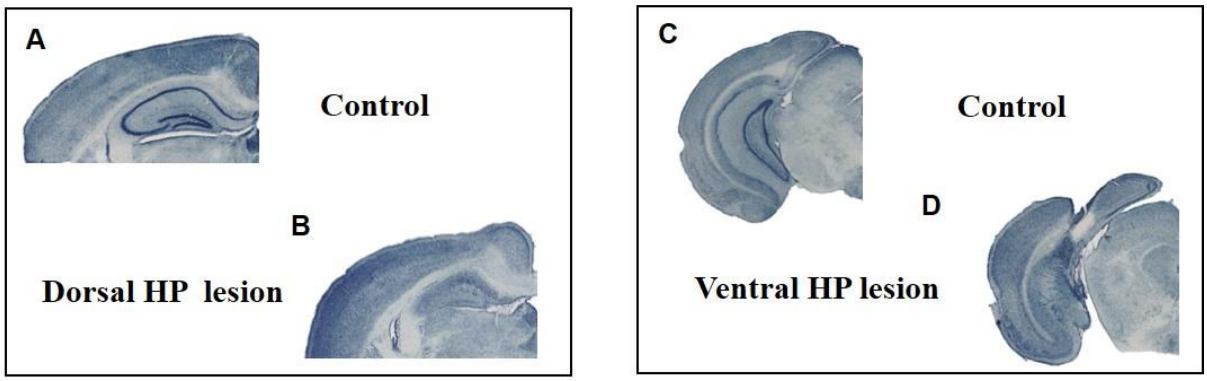


Fig. 26 Dorsal HP and ventral HP lesion. *Photomicrographs of Nissl-stained coronal sections of the dorsal HP lesion compared to respective control (**A-B**) and the ventral HP lesion compared to respective control (**C-D**).*

Anteroposterior analysis of the dorsal HP lesion, relative to bregma (Franklin and Paxinos, 2001) showed that animals had a wide bilateral lesion which extended from the coordinates -1.22 to -2.18, with the most extended lesion including wide neuronal loss and gliosis, evident with Nissl method, in CA1, CA2 and CA3 fields of the HP and dentate gyrus, while the smallest lesion covers CA1, CA2 and CA3 with no dentate gyrus (Fig. 27 A). Analysis of stained tissue-sections did not reveal damage to either extraHP structures or neocortex in the vicinity of the lesion except for the mechanical damage caused by the needle insertion. This is consistent with the pattern of the damage produced by the dorsal HP injection of NMDA at our same concentration (20µg/µl) in rats reported by Maren S. et al., 1997(Maren, Aharonov et al. 1997). The antero-posterior analysis of the ventral HP lesion revealed that the lesion extended from -2.70 to -3.64 from bregma with the most extended lesion including CA1, CA2 and CA3 and dentate gyrus, while the smallest lesion covered a portion of the CA1, CA2 and CA3 and a small portion of the dentate gyrus (Fig. 27 B). Some of ventral HP lesioned

subjects showed partial damage to the tip of CA3 region of the very posterior end of the dorsal HP, but the extent of damage was minimal (data not shown). A minimum extension of the damage to the temporal part of the dorsal HP was reported in Pothuizen et al. 2004 and in Taejib Yoon and Tim Otto, 2007(Yoon and Otto 2007). In these the largest lesion in both the dorsal HP and the ventral HP lesion groups had an extensive bilateral damage throughout the entire HP region. Our lesion placement is comparable to these previously described.

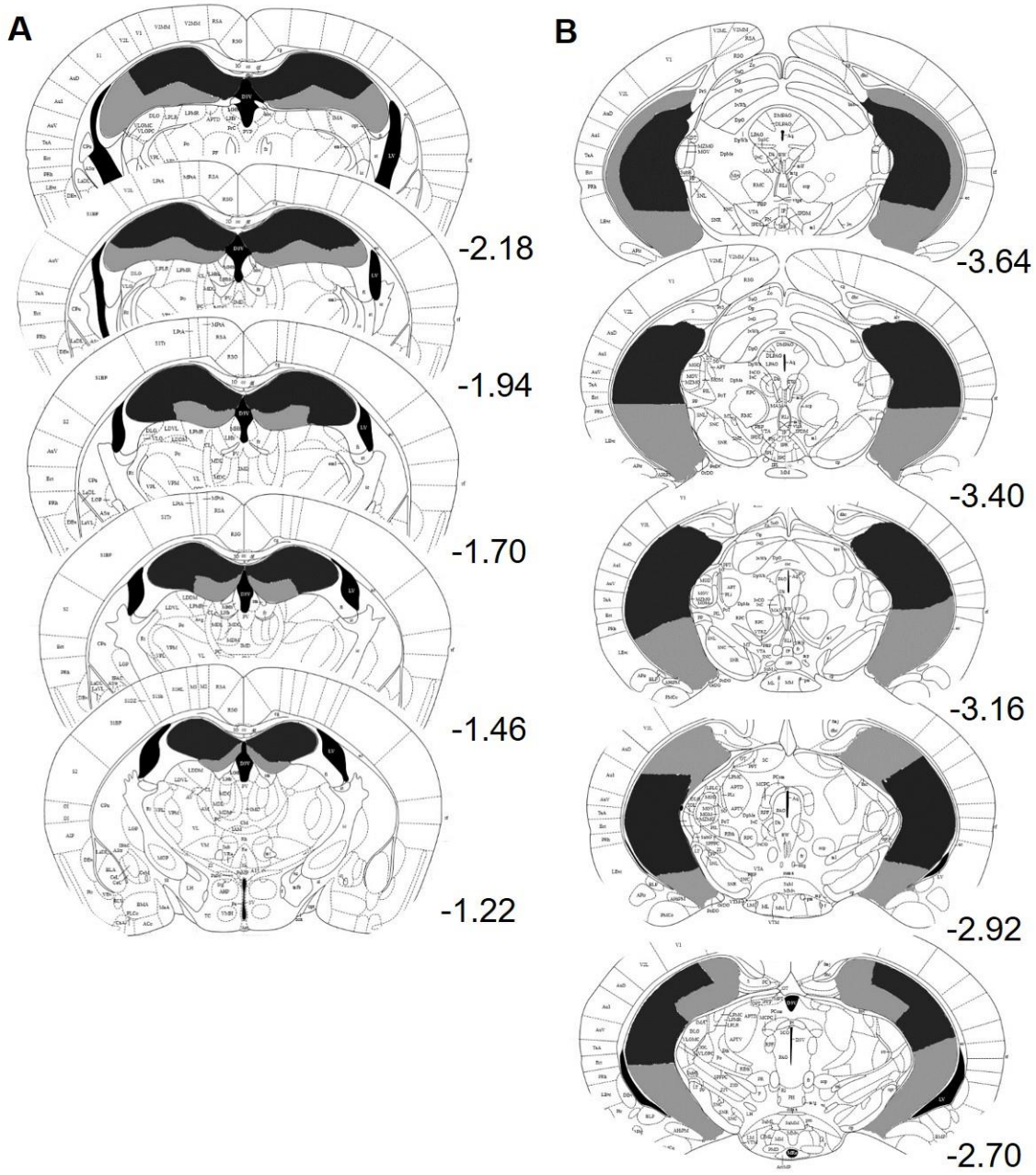


Fig. 27 Dorsal HP and ventral HP lesion. *Graphic reconstruction illustrating the largest (grey areas) and the smallest (black areas) lesion extent for dorsal HP lesion group (**A**) and ventral HP lesion group (**B**). The*

numerical values indicate the distance in millimeters relative to bregma in the anterior-posterior plane (Franklin and Paxinos, 2001).

Dorsal hippocampus, but not ventral hippocampus regulates object working memory capacity in mice

Here, we replicated the results found in Sannino et al., 2012 with the dorsal HP lesion and extended the study to the investigation of the role of the ventral HP in object WMC.

Animals with lesion to the dorsal and ventral HP and a group with sham lesion (control group) were subjected to the 6 DOT and the 6 IOT task with 1 min delay to evaluate object WMC. Duncan *post-hoc* analysis on objects exploration during the test phase of the 6 DOT revealed that both control and ventral HP lesion groups explored the new object significantly more than the other 5 familiar objects, while dorsal HP lesion group did not (Fig. 28 A). HP lesion did not affect neither the object exploration during the T2 phase (one-way ANOVA, treatment $F_{(2,23)} = 2.155$; $p = 0.1837$) nor the distance travelled in the arena in the T1 phase (one-way ANOVA, treatment $F_{(2,23)} = 1.614$; $p = 0.2209$) (Fig. 28 B-E). In the 6 IOT in all three groups the new object was preferred over the familiar ones (Fig. 27 8). Also in this case neither the objects exploration during the T2 phase (treatment $F_{(2,23)} = 0.640$; $p = 0.5365$) (Fig. 28 D) nor the distance travelled in the arena revealed any difference between groups (one-way ANOVA, treatment $F_{(2,23)} = 0.589$; $p = 0.5628$) (Fig. 28 F).

These results confirmed the previous results found in Sannino et al., 2012 about the involvement of the dorsal HP in object WMC and demonstrated that the ventral HP is not necessary for this task.

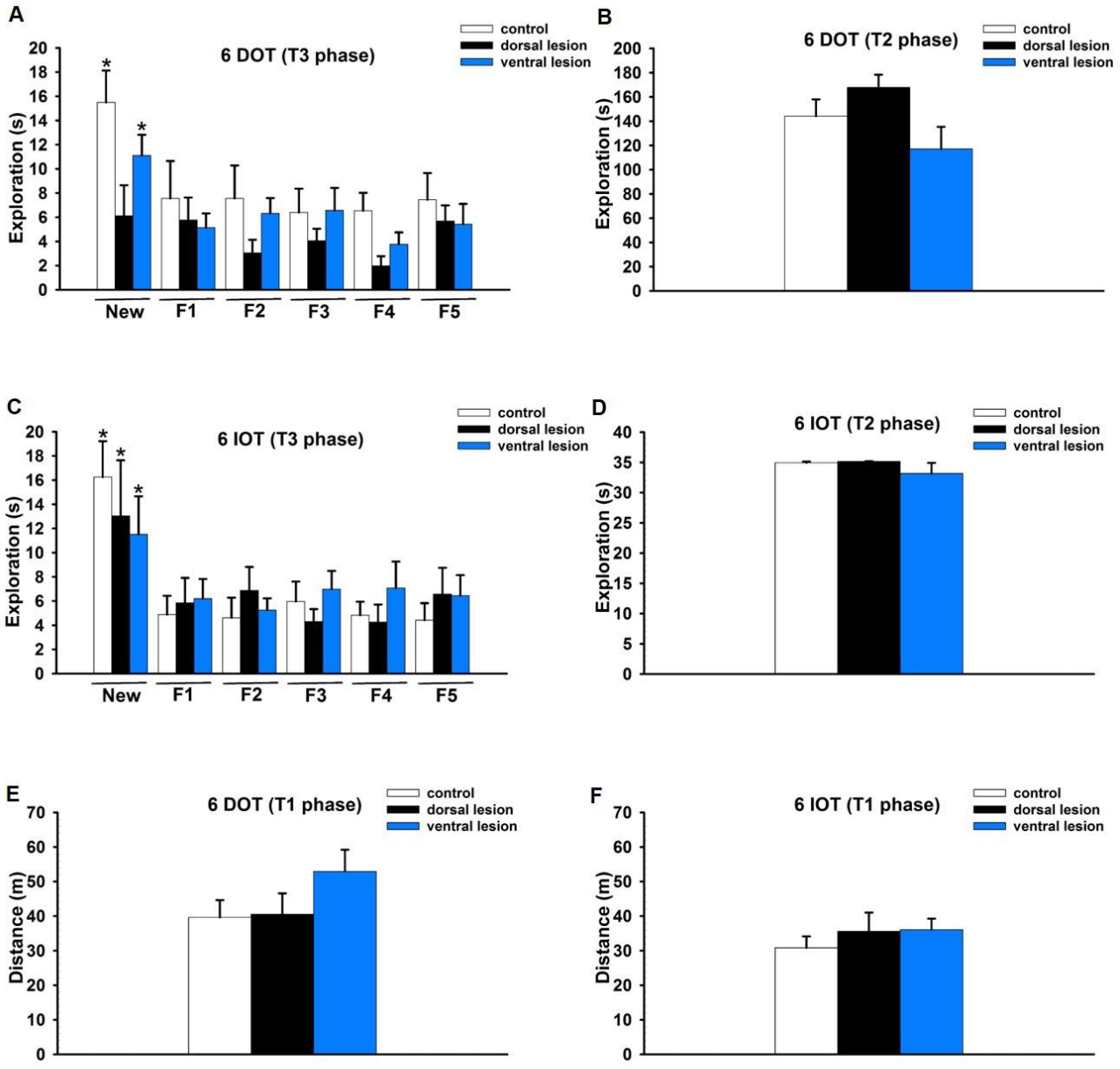


Fig. 28 Dorsal HP, but not ventral HP lesion impaired new object discrimination in the 6 DOT. *New and familiar objects (F1, F2, F3, F4, F5) exploration during the T3 phase (**A-C**) in control, dorsal HP and ventral HP lesion groups in the 6 DOT and in the 6 IOT. Total exploration of the objects during the T2 phase in control, dorsal HP and ventral HP lesion groups in the 6 DOT and in the 6 IOT (**B-D**). Distance travelled in the arena in the T1 phase of the 6 DOT (**E**) and 6 IOT (**F**) in control, dorsal HP and ventral HP lesion groups. Data are expressed as mean ±SEM. * p < 0.05 new vs. all of the other familiar objects; Duncan post-hoc analysis.*

Both dorsal hippocampus and ventral hippocampus are involved in allocentric spatial working memory capacity, but the ventral hippocampus is also crucial for egocentric spatial information processing

Although the dorsal HP has been more associated to spatial learning, whereas the ventral HP to non-spatial aspects of HP-dependent learning (Moser, Moser et al. 1993), the role of these two HP subregions in spatial WMC has never been addressed before. To address this issue we have used a modified version of the radial arm maze where the memory load was manipulated by allowing the animals to retrieve food from 3, 6 and 8 open arms.

The test starts with the PT phase (2 open and baited arms) where the animals are trained to the mechanism of the task. They learn that one piece of food is located at the end of each arm. If they re-enter in the same arm, the food is no longer present. Mean number of errors of control, dorsal HP and ventral HP lesion groups in this phase was analysed using a one-way ANOVA. Any significant difference between the three groups was found (treatment $F_{(2,27)}=2.575$; $p = 0.0947$) (Fig. 29 A).

In the next days, control, dorsal HP and ventral HP lesion groups were exposed to the ***WMC training phase*** where the number of open and baited arms changed from 3, 6 and 8 between trials and among the training days. The test was first performed with a confinement procedure (first 4 days), which was aimed at preventing the use of egocentric strategies; the next four days the confinement was removed to evaluate the use of egocentric strategy and the switch between the two.

The three-way ANOVA for repeated measures with all three groups (control, dorsal HP and ventral HP lesion) as between variable, and the number of open arms and procedures (confinement and no-confinement) as within variables, on the mean number of errors revealed that animals increased the number of errors when the number of open arms was increased; however, this effect was much more evident in the confinement than in the no confinement procedure (number of open arms $F_{(2,50)} = 152.107$; $p<0.0001$, procedure $F_{(1,25)} = 117.559$; $p<0.0001$, number of open arms x procedure $F_{(2,50)} = 21.971$; $p<0.0001$), as confirmed by post- hoc analysis (Fig. 29 B).

HP lesion impaired performance depending on the number of open arms and on the procedure (treatment $F_{(2,25)} = 7.137$; $p=0.0035$, treatment x procedure $F_{(1,25)} = 6.552$; $p=0.0052$, treatment x number of open arms $F_{(4,50)} = 5.061$; $p=0.0017$). To analyse separately the effects of the confinement and no-confinement procedure, a two-way ANOVA on two procedures was performed. HP lesion caused an impairment on the performance in the confinement procedure (treatment factor, $F_{(2,27)} = 7.357$; $p = 0.0028$). Duncan *post-hoc* analysis showed that control, dorsal HP and ventral HP lesion

groups made a mean number of errors with 8 open arms significantly major than with 3 and 6 open arms suggesting that the mean number of errors was memory load-dependent. Moreover, it revealed that both dorsal HP and ventral HP lesion groups made more errors than control group with 8 open arms. In the no-confinement procedure, HP lesion caused an impairment in the performance depending on the number of open arms (treatment $F_{(2,25)} = 11.468$; $p = 0.0003$; number of open arms $F_{(2,50)} = 55.008$; $p < 0.0001$; treatment x number of open arms $F_{(4,50)} = 5.732$; $p = 0.0007$). Duncan *post-hoc* analysis revealed that ventral HP lesion group made a mean number of errors significantly higher than control and dorsal HP lesion group with both 6 and 8 open arms (Fig. 29 B).

Finally, the evaluation of the time to complete the task across days showed that HP lesion did not affect this parameter. No treatment effect was found in a three-way ANOVA for repeated measures with control, dorsal HP lesion and ventral HP lesion groups as between factor (treatment $F_{(2,25)} = 2.286$; $p = 0.1225$). Neither a treatment effect was evident in a two-way ANOVA performed separately on the confinement procedure with all three groups (treatment $F_{(2,27)} = 2.472$; $p = 0.1034$) nor in the no-confinement procedure (treatment $F_{(2,25)} = 1.363$; $p = 0.2742$) (Fig. 29 C).

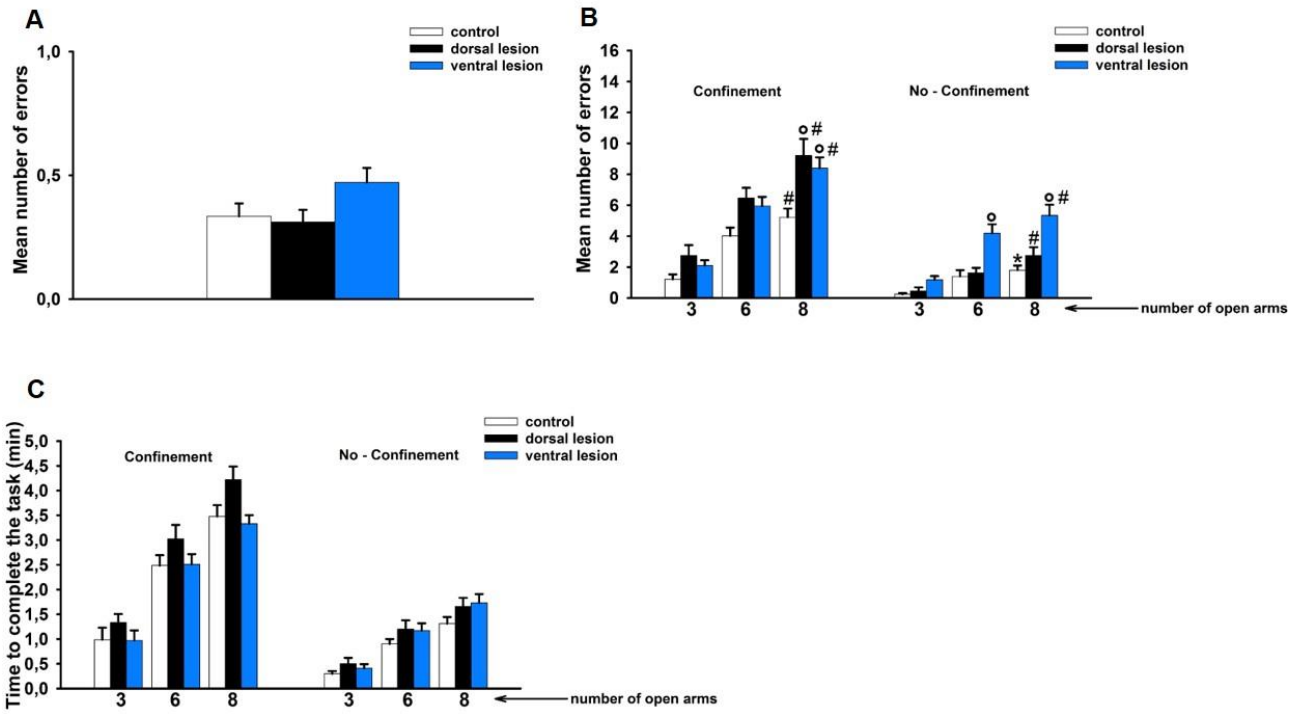


Fig. 29. Both dorsal HP and ventral HP lesion impaired performance in the confinement procedure in highest memory load conditions, but only ventral HP lesion impaired performance in the no-confinement procedure in high memory load conditions. *Mean number of errors in the PT phase in control, dorsal HP lesion and ventral HP lesion group (**A**). Mean number of errors in WMC training phase with the confinement and no-confinement procedure in control, dorsal HP and ventral HP lesion groups (**B**). Time to*

*complete the task in control, dorsal HP and ventral HP lesion groups (**C**). Data are expressed as mean ± SEM. * p < 0.05 vs. 3 arms, within group, within procedure; # p < 0.05 vs. 6 arms, within group, within procedure; ° p <0.05 dorsal HP and ventral HP lesion group vs. control group. Duncan post - hoc analysis.*

All together these results showed that both dorsal HP and ventral HP lesion groups were impaired in high memory load conditions in the confinement procedure but only the ventral HP lesion group was impaired in high memory load conditions in the no- confinement procedure. Therefore, we can conclude that both dorsal HP and ventral HP lesion caused a defect in spatial WMC as evidenced in the confinement procedure. In the no-confinement procedure, where the animals were free to explore the maze and egocentric strategies could be acquired, only the ventral HP lesion group was impaired. The analysis of the time to complete the task demonstrated that the defect found in dorsal HP and ventral HP lesion groups was a WMC defect and It did not depend on others parameters (Fig. 29 C).

Ventral hippocampus has a crucial role in regulating the use of a non-spatial sequential strategy in a no-confinement procedure of the working memory capacity radial arm maze task.

Olton and Samuelson (1976) (Olton and Samuelson, 1976) demonstrated that rats solved the eight-arm radial maze test by identifying each of the different spatial locations and remembering which ones had been chosen. However, there are several ways of simplifying the task and reducing the information that must be maintained in memory in order to perform accurately. One such strategy would be to use non-spatial strategies that are egocentric strategies through which subjects repeatedly choose arms at fixed distance apart. They are adopted by rodents when they are free to explore a maze as the radial arm maze in a full-baited condition (Bolhuis, Bijlsma et al. 1986). Based on these evidence, in the no-confinement procedure we analysed the "sequential strategy" (see methods and materials), using the RAM. A three-way ANOVA for repeated measure with control, dorsal HP and ventral HP lesion groups as between variable was performed to evaluate the use of this strategy in the confinement and no-confinement procedure. The use of the sequential strategy depended on the procedure (procedure $F_{(1,25)} = 168.124$; $p < 0.0001$), on the number of open arms (number of open arms $F_{(2,50)} = 27.571$; $p < 0.0001$) and on their interaction (procedure x number of open arms $F_{(2,50)} = 123.306$; $p < 0.0001$). HP lesion impaired the use of the sequential strategy (treatment $F_{(2,25)} = 8.272$; $p = 0.0017$) depending on the procedure (treatment x procedure $F_{(2,25)} = 12.163$; $p = 0.0002$) and on the number of open arms (treatment x number of open arms $F_{(4,50)} = 5.431$; $p = 0.0010$). A separate two-way ANOVA on the confinement and no-confinement procedure was also performed to

better dissociate the effects of the HP lesion in the two procedures. No effect of the treatment was found in the confinement procedure (treatment $F_{(2,27)} = 0.243$; $p = 0.7862$), while in the no-confinement procedure HP lesion affected the use of the sequential strategy depending on the number of open arms (treatment $F_{(2,25)} = 11.315$; $p = 0.0003$; number of open arms $F_{(2,50)} = 80.381$; $p < 0.0001$; treatment x number of open arms $F_{(4,50)} = 10.281$; $p < 0.0001$). In the confinement procedure, control, dorsal HP and ventral HP lesion groups had a low similar sequential score with 3, 6 and 8 open arms. This demonstrates that the confinement procedure prevented the use of the sequential strategy. In the no-confinement procedure, Duncan *post-hoc* analysis revealed that control and dorsal HP used the sequential strategy with 8 open arms more than with 6 and 3 open arms. This suggested that the full baited condition is the best condition where to use the sequential strategy to perform accurately the task. Dorsal HP lesion group was impaired in the use of the sequential strategy as compared to control only when animals were confronted with 8 open and baited arms while ventral HP was impaired in the use of the sequential strategy with both 6 and 8 open and baited arms (Fig. 30 A).

All together these results showed that our confinement (5 sec) procedure is useful to prevent the use of the sequential strategy, as demonstrated by very low score in control animals. Removing the confinement, control animals promptly switched to the sequential strategy to solve the task. Animals with dorsal HP lesion were almost comparable to control, except for reducing the use of the sequential strategy when confronted with 8 arms in the no confinement procedure. In contrast, animals with ventral HP lesion were significantly impaired the use of this strategy compared to the control and the dorsal HP group.

Then, we measured the "alternating strategy" (see methods and materials), that is the tendency to skip one arm to enter in the next one. We performed a three-way ANOVA to analyse the use of the alternating strategy in the confinement and no- confinement procedure with control, dorsal HP and ventral HP lesion groups as between variable and the number of open arms and the procedure as repeated measure. HP lesion affected the use of the alternating strategy (treatment $F_{(2,25)} = 9.980$ $p = 0.0007$) depending on the procedure (treatment x procedure $F_{(2,25)} = 5.539$; $p = 0.0102$) and on the number of open arms (treatment x number of open arms $F_{(4,50)} = 6.068$; $p = 0.0005$). We also performed a two-way ANOVA on both the confinement and no-confinement procedure. HP lesion did not produce any effect in the confinement procedure (treatment $F_{(2,27)} = 2.292$; $p = 0.1204$). The alternating strategy score was always 0 with 3 open arms in the confinement procedure because the arms between the 3 open arms are closed so that the 3 open arms become consecutive. Control group made a mean score of 3.359±0.292 with 6 open arms and a mean score of 2.844±0.291 with 8 open

arms. Such score indicates that they did not use this strategy to solve the task in the confinement procedure. In the no-confinement procedure, HP lesion affected the use of the alternating strategy depending on the number of open arms (treatment $F_{(2,25)} = 12.055$; $p = 0.0002$). Duncan *post-hoc* analysis showed that ventral HP lesion group compensated for the impaired use of the sequential strategy, using the alternating strategy more than the control group with 6 and 8 open arms and more than the dorsal HP lesion group with 8 open arms (Fig. 30 C).

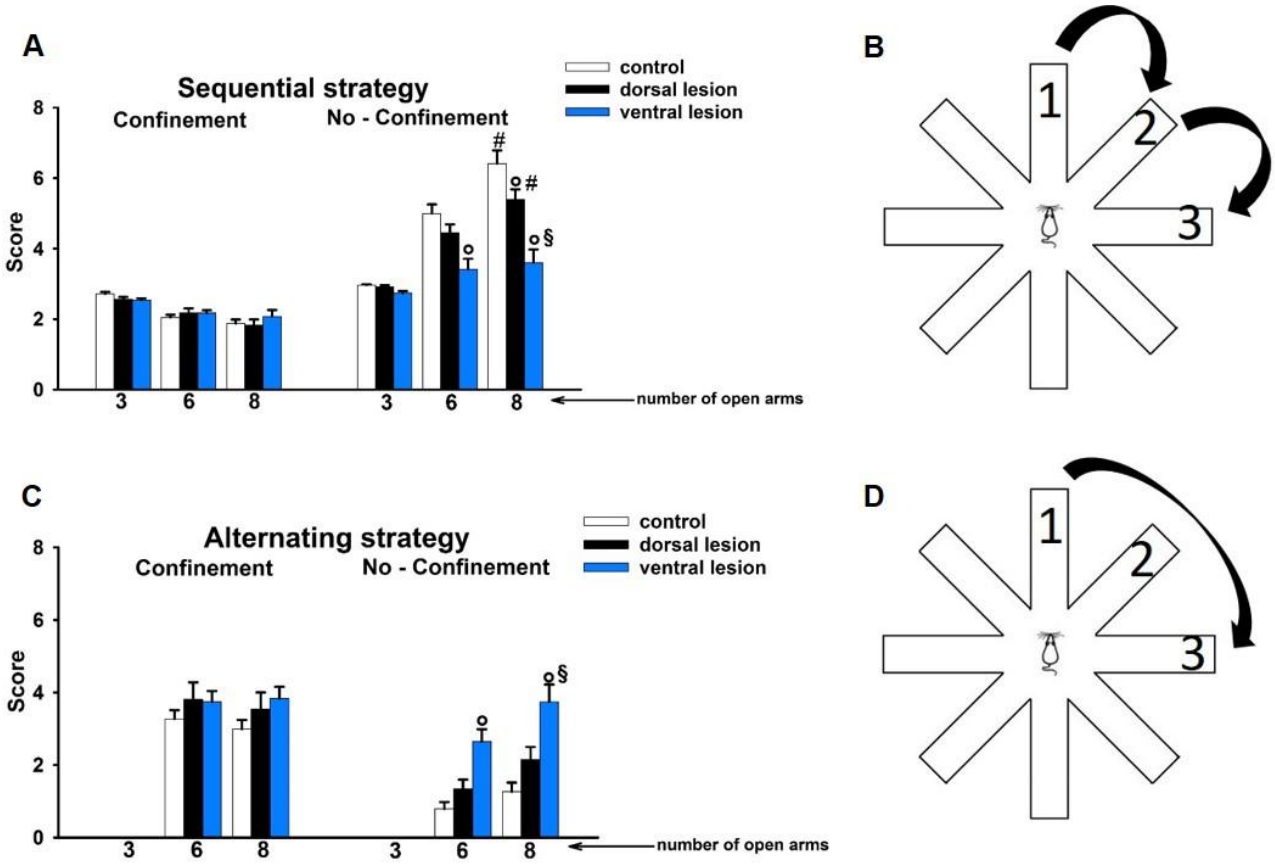


Fig. 30 Ventral HP more than dorsal HP regulates the use of the sequential strategy in a no-confinement procedure of the radial arm maze task. *Score of the sequential strategy made by control, dorsal HP and ventral HP lesion groups in the confinement and no-confinement procedure (**A**). Schematic representation of an example of sequential strategy (**B**). Score of the alternating strategy made by control, dorsal HP and ventral HP lesion groups in the confinement and no-confinement procedure (**C**). Schematic representation of an example of alternating strategy (**D**). Data are expressed as mean ± SEM. # p<0.05 8 vs. 6 open/baited arms within group, within procedure; ° p<0.05 vs. control. § p <0.05 vs. dorsal lesion. Duncan post – hoc analysis.*

Then, in order to measure the learning of the sequential strategy in the switch from the confinement procedure to the no-confinement procedure, a two-way ANOVA analysis for repeated measures on the last day of confinement and the four days of no-confinement was performed. The two-way ANOVA analysis performed on control, dorsal HP and ventral HP lesion groups revealed an effect

of days ($F_{(4,100)} = 19.701$; $p < 0.0001$ (6 open arms); $F_{(4,100)} = 34.501$; $p < 0.0001$ (8 open arms)). In fact, Duncan *post-hoc* analysis showed a significant increase in the use of the strategy in day 1 of the no-confinement procedure in all three groups with 8 open arms (Fig. 31).

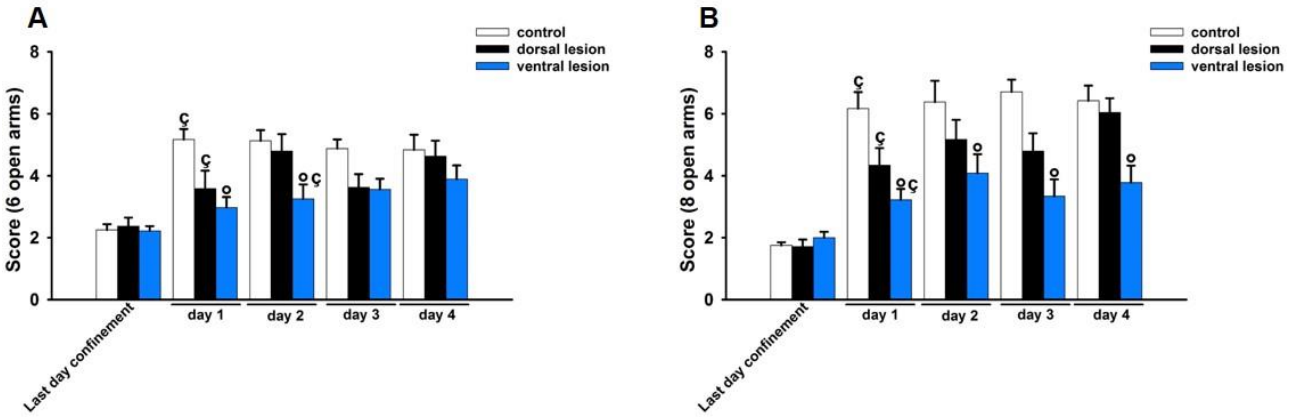


Fig. 31 Control, dorsal HP and ventral HP lesion groups acquired immediately the sequential strategy in a no-confinement procedure. *Score of the sequential strategy with 6 open arms (**A**) and 8 open arms (**B**) in control, dorsal HP and ventral HP lesion groups in the switch from the confinement procedure to the no-confinement procedure. Data are expressed as mean± SEM. ° p < 0.05 vs. control; ç p < 0.05 vs. last day confinement. Duncan post-hoc analysis.*

On the basis of this last analysis, we can add that control, dorsal HP and ventral HP lesion groups immediately acquired the sequential strategy when they switched from a confinement procedure to a no-confinement procedure suggesting that it is an innate behaviour in conditions of free exploration.

In conclusion, these results reported that with a confinement procedure in the radial arm maze task, control, dorsal HP and ventral HP lesion groups did not adopt an egocentric strategy to solve the task, but they made some entries in the consecutive arms and others in the alternate arms. The removal of animal confinement in the center of the maze before an arm choice favoured the use of the sequential strategy in the control group and in the dorsal group. In contrast, HP lesion impaired the switching to the sequential strategy in the ventral HP group, and affected its optimal use in the dorsal HP group. Consequently, ventral HP lesion group used the alternating strategy more than control and dorsal HP lesion groups.

In a no-confinement procedure, not preceded by a confinement procedure, both dorsal and ventral hippocampal lesion impaired performance

The impairment in the no-confinement procedure observed in ventral HP lesion group could be due to an impaired ability to switch between procedures or to a defect in the acquisition of the sequential strategy. In order to answer this issue, we tested other control (n = 13), dorsal HP lesion (n = 10) and ventral HP lesion groups (n= 9) in 4 days-WMC radial maze task with the no-confinement procedure, not preceded by the confinement procedure. First of all, we measured mean number of errors using a two-way ANOVA for repeated measures with the number of open arms as within variable and control, dorsal HP and ventral HP lesion groups as between variable. HP lesion affected the performance (treatment $F_{(2,29)} = 7.163$; $p = 0.0030$) depending on the number of open arms (treatment x number of open arms $F_{(2,58)} = 5.401$; $p = 0.0009$). Duncan *post-hoc* analysis revealed that the behaviour of groups perfectly overlapped that of the three groups tested in the no-confinement after being submitted to the confinement procedure. The only difference was that dorsal HP lesion group was more impaired when tested with 8 open arms in this new condition (Fig. 32).

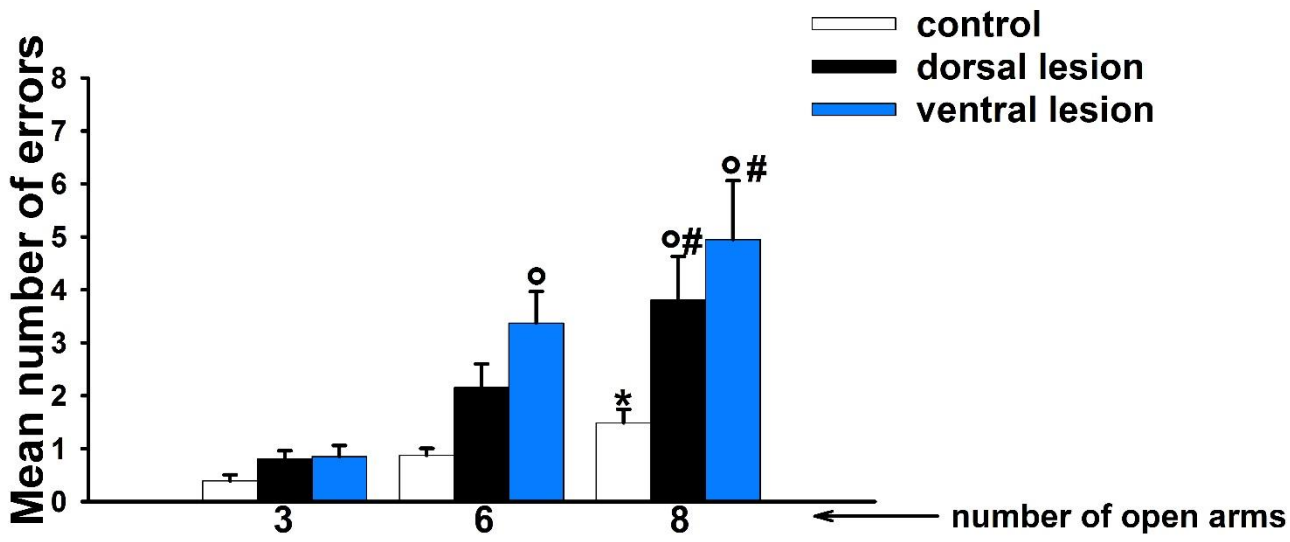


Fig. 32 Both dorsal HP and ventral HP lesion impaired performance in a no-confinement procedure of the WMC radial arm maze task, not preceded by a confinement procedure. *Mean number of errors in a no-confinement procedure of the WMC radial arm maze task, not preceded by a confinement procedure in control, dorsal HP and ventral HP lesion groups. Data are expressed as mean ± SEM. * p < 0.05 vs 3 arms, within group; # p < 0.05 vs 6 arms, within group; ° p < 0.05 vs control, within arm. Duncan post - hoc analysis.*

Both dorsal hippocampal and ventral hippocampal lesion impaired the use of the sequential strategy in a no-confinement procedure, not preceded by a confinement procedure

As reported above, the animals spontaneously tend to use egocentric strategies when they are free to explore the maze. Considering that the most used strategy when the animals are free to explore the maze (no-confinement procedure) is the sequential strategy, the scores for this strategy were measured across all groups.

We performed a two-way ANOVA for repeated measures with control, dorsal HP and ventral HP lesion groups as between variable. HP lesion impaired the use of the sequential strategy depending on the number of open arms (treatment $F_{(2,29)} = 6.319$; $p = 0.0053$; number of open arms $F_{(2,58)} = 98.686$; $p < 0.0001$; treatment x number of open arms $F_{(4,58)} = 5.490$; $p = 0.0008$). Duncan *post-hoc* analysis confirmed the results obtained in the previous conditions, showing a major impairment in the use of the sequential strategy in the ventral HP lesion group, and a mild impairment in the dorsal HP lesion group when confronted with 8 baited arms. As well as for the sequential strategy, we performed a two-way ANOVA with all three groups as between variable on mean score of the alternating strategy. HP lesion affected the use of the alternating strategy (treatment $F_{(2,29)} = 4.960$; $p = 0.0140$). Interestingly, Duncan *post-hoc* analysis revealed that both dorsal HP and ventral HP lesion groups used the alternating strategy more than control with both 6 and 8 open arms (Fig. 33). Therefore, these results suggest that both dorsal HP and ventral HP lesion groups compensated for their impairment in the use of the sequential strategy, using the alternating strategy, when they were not previously trained to do not rely on the sequential strategy.

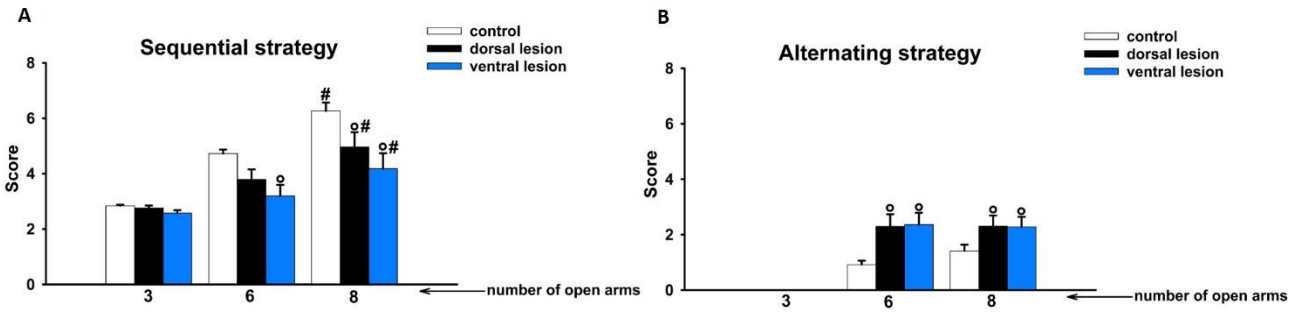


Fig. 33 Both dorsal HP and ventral HP lesion impaired the use of the sequential strategy in a no-confinement procedure, not preceded by a confinement procedure. *Score of the sequential strategy made by control, dorsal HP lesion and ventral HP lesion groups (**A**). Score of the alternating strategy made by control, dorsal HP and ventral HP lesion groups (**B**). Data are expressed as mean ± SEM. # p<0.05 8 vs 6 open/baited arms within group, within procedure; ° p <0.05 vs. control*

All together these results demonstrated that when control animals are tested in a no-confinement procedure, not preceded by a confinement procedure, they use the sequential strategy more than the alternating strategy. Both dorsal HP and ventral HP lesion impaired the use of the strategy sequential when the animals were confronted with 8 open arms. Ventral HP lesion also impaired this use with 6 open arms suggesting a more severe defect than in dorsal HP lesion. These results demonstrated that the impairment found in the ventral HP lesion group in the confinement and no-confinement procedure was not due to impaired switching ability. The alternating strategy was more used by both dorsal HP and ventral HP lesion groups compared to control group.

A classical ventral hippocampus-dependent paradigm: ventral hippocampus, but not dorsal hippocampus modulates anxiety-like behaviour

The current idea is that the dorsal HP, defined as 50% of HP volume starting at the septal pole (Bannerman, Yee et al. 1999) and referred to as posterior HP in primates (Strange, Witter et al. 2014**)** , has a preferential role in spatial learning and memory, and that the ventral HP, defined as 50% of HP volume starting at the temporal pole, and referred to as anterior HP in primates (Strange, Witter et al. 2014**)**, may have a preferential role in anxiety-related behaviours.

Here the contribution of the dorsal and ventral HP to anxiety-like behaviour was examined in an elevated plus maze. We tested the theory about the involvement of the ventral HP in emotional processes (McNaughton and Gray 2000) on our animals in order to verify if our lesions produced the same results found in literature.

We performed a one-way ANOVA on the percentage of open arms entries and percentage of open arms time with all three groups as between variable. The group difference was significant in both the percentage of open arms entries ($F_{(2,27)} = 4.438$; $p = 0.0215$) and percentage of open arms time (treatment $F_{(2,27)} = 3.863$; $p = 0.0335$). Duncan *post-hoc* analysis revealed that ventral HP lesion group had a significantly higher percentage of entries in the open arms and spent a higher percentage of time in the open arms than control animals. By contrast, there were no differences between dorsal HP lesion groups and control group (Fig. 34).

Thus, our dorsal HP and ventral HP lesions confirmed a role of the ventral HP in modulating anxiety-like behaviour (Bannerman, Grubb et al. 2003).

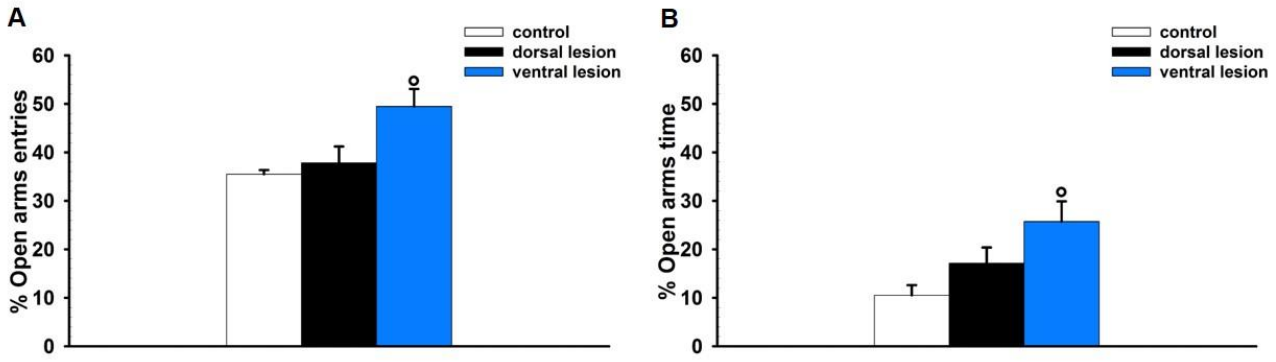


Fig. 34 Reduced anxiety-like behaviour in ventral HP lesion group. *Percentage of open arm entries (**A**) and percentage of open arm time (**B**) in control, dorsal HP and ventral HP lesion groups compared to control group. Data are expressed as mean ± SEM. ° $p < 0.05$ vs. control. Duncan post-hoc analysis.*

Discussion

In this study we found functional differences between the dorsal and ventral subregions of the HP in regulating object WMC but any difference was found in regulating allocentric spatial WMC. A major involvement of the ventral HP in regulating egocentric spatial memory was found. A recent study in CD1 mice reported a role for the dorsal HP in WM in high memory load (Sannino, Russo et al. 2012) confirming the results found in monkeys and in humans (Levy, Manns et al., 2003, Beason-Held, Rosene et al., 1999). Anatomic, genetic and behavioral studies suggested that the HP is subdivided into a dorsal and a ventral portion (Swanson and Cowan, 1977, Moser and Moser, 1993; Thompson and Pathak, 2008). Whether these two HP subregions differently regulate WMC has never been explorated. In this study we investigated if there is a functional differentiation between the dorsal and the ventral HP in regulating the limited capacity of WM using a neurotoxic selective dorsal and ventral HP lesion approach. First, we explorated the role of these two subregions in object WMC testing the dorsal HP and the ventral HP lesion groups in the 6 DOT described in Sannino et al., 2012. Here, we confirmed the role of the dorsal HP in object WMC and we did not find any involvement of the ventral part of the HP. Then, we investigated the role of these two regions in spatial WMC testing both lesion groups in a WMC version of the classical radial arm maze task using a modified protocol of the version described in Olivito et al., 2016. In our study we modulated the memory load by changing the number of open arms between 3, 6 and 8 open arms and exposing the mice to an allocentric version of the task (the confinement procedure) in the first 4 days and to a version where both allocentric and egocentric spatial memory can be used (the no-confinement procedure) in the last 4 days. In the confinement procedure the animals were confined in the central zone of the apparatus to prevent the use of the egocentric strategies that are otherwise developed when the animals are free to explore the maze (no-confinement procedure). In this procedure we found an impairment in both dorsal HP and ventral HP lesion groups in high memory load suggesting a complementary role of the dorsal and ventral HP in mediating allocentric spatial WMC. A dissociation between the two regions was found in the no-confinement procedure where the involvement of the ventral HP was major than the dorsal HP. This major involvement of the ventral HP is parallel to a major impairment in the use of the sequential strategy found in the ventral HP lesion group suggesting a crucial role for the ventral HP in mediating the acquisition of egocentric strategies to solve the task. A no- confinement procedure, not preceded by a confinement procedure confirmed the crucial role of the ventral HP in regulating the use of egocentric strategies. In a final analysis of the effects of our dorsal HP and ventral HP lesions on a classical task used in literature to

dissociate their function, we found the specific involvement of the ventral HP in mediating anxiety-like behavior confirming the results present in literature.

Dorsal hippocampus, but not ventral hippocampus mediates object working memory capacity

Although the HP has been traditionally associated to spatial memory, several studies in the past focused on non- spatial memory tasks (Aggleton, Hunt et al. 1986). Delayed non-matching to sample (DNMS) has been widely used in monkeys to assess non spatial HP function. On this one-trial recognition test, animals are required to discriminate familiar (previously seen) from unfamiliar (novel) stimulus objects. Although DNMS has become the currently accepted standard for assessing recognition memory in nonhuman primates, its sensitivity to HP dysfunction is controversial. In a series of studies using DNMS it was concluded that when damage is restricted to the HP monkeys are not impaired in their ability to recognize previously presented stimuli but it is necessary that both the HP and the amygdala are damaged to found the defect (Mishkin 1978). In other studies, on the contrary the HP alone produced the impairment in recognition memory (Zola-Morgan and Squire 1986). In rats, damage to the ventral temporal region, including the HP, had no lasting effect on trial-unique DNMS. Object recognition performance of all animals was comparable to that of normal rats when tested with retention intervals up to 30 s. Defects in object recognition memory was found only with delays over 60 sec (Rothblat and Kromer 1991). These results were explained by the easy processing of the task that could be solved using only a judgement of familiarity of the previously experienced stimulus and this could be fully mediated in regions of sensory and/or association neocortex without the involvement of the HP (Wan, Aggleton et al. 1999).

Recent studies in human and non- human primates demonstrated an involvement of the HP in items WM only when the number of items to remember is high.

Amnesic patients with bilateral damage to the HP region were impaired in recognition span tasks. Each task began by presenting one item, then two items, then three items, and so on, and the patients had to select the novel item (Levy, Manns et al. 2003). The same impairment was found in patients with Alzheimer's disease, Huntington's disease or Korsakoff's syndrome (Moss, Albert et al. 1986). When the same test was adapted for monkeys, monkey with bilateral lesions of the HP formation were impaired on span tests for spatial location, colors, and objects (Beason-Held et al., 1999).

In rats there is little experimental evidence in support of the role of HP in WM span. In fact, there is a study reporting that complete HP lesions impaired performance in a spatial span task but not in an

odour recognition span task (Dudchenko, Wood et al. 2000). Our group, using the DOT/IOT task showed that mice with lesions in the dorsal region of the HP were impaired in novel object discrimination in high memory load condition (6 DOT). On the contrary, when dorsal HP lesioned animals were tested in 3 or 4 DOT, they were not impaired, suggesting that the recruitment of the dorsal HP in object recognition memory depends on the stimulus set size. A low memory load condition can be processed by the perirhinal cortex while a high memory load condition requires the recruitment of the HP (Sannino, Russo et al., 2012). Rats with excitotoxic dorsal HP lesions were not impaired in a novelty object detection task with only two objects (Hoge and Kesner 2007) confirming the results found in Sannino et al., 2012 about a lack of involvement of the dorsal HP in low memory load. In the present study we replicated the results obtained by Sannino and colleagues in 2012 in the same laboratory.

Then we extended these results to the investigation of the role of the ventral region of HP in this process in order to confirm or disprove a functional dissociation between these two regions in item WMC. Our results showed that ventral HP is not recruited in different object recognition task in high memory load conditions, because ventral HP lesion group was able to discriminate the new object from the others 5 familiar objects in the 6 DOT. No evidence is present in literature regarding the involvement of the ventral HP in object memory span. Here we found a dissociation between the dorsal and the ventral HP in the 6 DOT that could be explained by the different anatomical connectivity. Neocortical areas (sensitive and visual areas) convey their projections, through the perirhinal and post-rhinal cortices, predominantly to the dorsal HP (Burwell and Amaral 1998). Considering that the perirhinal cortex is essential for the object recognition memory (Barker, Bird et al. 2007), the major projections from this region to the dorsal HP could account for an involvement of the dorsal HP, and not of the ventral HP. In fact, in a recent study rats with lesions in the lateral entorhinal cortex, preferentially connected with the dorsal HP (Strange, Witter et al. 2014), were impaired in the objects exploration task with 4 different objects demonstrating a role for the lateral entorhinal cortex in the objects processing. Conversely, rats with lesions in medial entorhinal cortex, connected to ventral HP (Strange, Witter et al. 2014), were not impaired in the object exploration task but only in spatial tasks (Van Cauter, Camon et al. 2013).

Egocentric, but not allocentric, information load dissociates the role of the dorsal and ventral hippocampus in spatial WMC.

The contribution of the HP to spatial WMC has been investigated by different laboratories, which consistently reported, in rats and monkeys, a performance above chance levels when hippocampectomized animals were required to remember a single cup location, but an impairment when they had to remember more (Dudchenko et al. 2000, Angeli, Murray et al. 1993).

How HP lesions can affect spatial WMC in the radial arm maze task have not been examined. Much research focused on the differential involvement of the HP in a version of the radial arm maze task where some arms were baited (working memory component) and others were always unbaited (reference memory) (Olton et al., 1979).

When it became clear that the HP may not constitute a functionally uniform structure, and that a functional dissociation exists along its septo-temporal axis, a four-baited/four-unbaited eight-arm radial maze procedure (Jarrard 1983) was used to test the effects of dorsal and ventral HP lesions on working memory component and reference memory component of the task. Dorsal HP but not ventral HP lesions led to significant impairments in spatial reference and working memory, according to the theory of a functional dissociation between dorsal and ventral HP with dorsal HP preferentially involved in spatial memory (Pothuizen, Zhang et al. 2004). On the contrary, a similar c-fos (an immediate early gene used as marker of cell activity) expression was found in dorsal and ventral HP after a standard eight arms radial arm maze; nevertheless, a higher involvement of dorsal HP was found when the final day of the test was performed in a different room suggesting a crucial role for the dorsal HP in the initial encoding of the room cues (Vann et al., 2000).

Although, none of these studies specifically manipulated the information load and controlled for the use of sequential strategy, the results obtained are generally in line with our findings in showing that in conditions of high load (8 baited arms) both the ventral and the dorsal HP are involved in WM, but the ventral HP might be regulating non spatial aspects of the task.

The radial arm maze was used to assess WMC first in Olton (1977), then in Tarantino et al. (Tarantino, Sharp et al. 2011) where a 17 arms and a 12 arms radial arm maze were used, respectively. In Olivito et al., 2016, an 8 arms radial arm maze was used to modulate the memory load by increasing the number of open and baited arms. During training, they were subjected to 10 different trials each day, with a random number of open and baited arms (3, 6 or 8 open). A significant effect of number of open arms was observed in control mice (C57BL/J mice) with a drop of the performance with 6 and

8 open arms compared to 3 open arms suggesting the validity of this paradigm to tap spatial WMC. No use of the sequential strategy was found in this mouse strain.

Although, no use of the sequential strategy was found in this mouse strain, other mouse or rat strains develop egocentric strategies that is a way to lower WM load. Olton (1977) demonstrated that when rats are left to freely explore and solve the eight arm radial maze, they exhibited two general response tendencies, consistently turning clockwise or counterclockwise when entering the center platform after choosing an arm (equivalent of our sequential strategy), and skipping at least one but usually not more than four arms before making their next choice. These response tendencies would bring to a simplification of the task. In Olton's study (1977) the animals were confined for about 15 seconds after every choice, in a so-called confinement procedure in order to interfere with these and any other ongoing response tendencies. An analysis of response choices indicated that the confinement procedure effectively disrupted the response patterns.

In order to avoid that the use of these non-spatial strategies interferes with our study of the spatial WMC, we used the WMC procedure of the radial arm maze task used in Olivito et al., 2016 but we introduced a 5 seconds confinement between choices (confinement procedure). The extremely low score of the sequential strategy suggested that this procedure is sufficient to interrupt the use of the sequential strategy. We found that the performance (mean number of errors) was memory-load dependent with a mean number of errors with 6 and 8 open arms major than with 3 open arms, in both control and lesioned animals. According to the hypothesis of a role of the HP in WM in high memory load conditions, both dorsal and ventral HP lesions had effect on the performance only in high memory load conditions (8 open arms). Therefore, the defect found in high memory load is a spatial WMC deficit. Other factors as the time to complete the task did not show group differences as reported in Olivito et al., 2016.

When the same animals were tested in a no-confinement procedure of the radial arm maze task, the use of the sequential strategy was immediately evident in control animals confirming a previous study where animals tested first in the confinement procedure and then tested in a no-confinement procedure, immediately acquired the sequential strategy demonstrating that it is the preferential strategy in this condition (Dubreuil, Tixier et al. 2003). In Dubreuil's study rats tested in a no-confinement procedure of the radial arm maze exhibited a degree of divergence equal to 7, where one degree of divergence corresponded to the transition to the adjacent arm, and 7 degrees of divergence corresponded to the transition to 7 consecutive arms. In contrast, in a 10 sec confinement procedure, rats never exhibited degree of divergence equal to 7. In the no confinement procedure rats

demonstrated a preference for a sequential or clockwise strategy while in the confinement procedure they do not show any preference.

We demonstrated that when control animals are first tested in a no-confinement procedure, they acquire the strategy in the second day of the test (data not shown). This confirms the hypothesis that the use of the sequential strategy seems an innate behaviour rather than an acquired one. When all arms are baited, rats use this strategy as the best strategy (Bolhouis and Bijlsma, 1986, Crusio and Schwegler, 1987, Dale and Innis, 1986).

On the contrary, both dorsal HP and ventral HP lesion groups were impaired, ventral HP more than dorsal HP lesion groups, although the impairment in ventral HP lesion group was less severe than that reported in the confinement and no- confinement procedure probably for the major complexity associated in that case to the switch from one procedure to one other. Both HP lesion groups compensated for the deficit in the use of the sequential strategy using another strategy that we called alternating strategy. A study in literature is consistent with our data showing that HP lesioned animals employed a sequence of 90° (angle between two arms) that is the equivalent of our alternating strategy (Okaichi and Oshima,1990).

The major defect in the use of the sequential strategy found in the ventral HP lesion group in the switch from the confinement to the no-confinement procedure correlated with the major mean number of errors found in the ventral HP lesion groups confirming that the sequential strategy is the best strategy in this conditions to accurately perform the task. This major defect found in the use of the sequential strategy is consistent with previous findings showing *c-fos* activation in the ventral CA1 subfield in animals trained to use an egocentric strategy in a starmaze task (Fouquet and Babayan, 2013). It has also been demonstrated that ventral HP has a role in temporal order memory, the capacity to distinguish between two spatial localizations visited at different points in the time (Wong, Howland et al., 2007).

The reliance of the clockwise strategy on motor programming was previously hypothesised by Ammassari-Teule and Caprioli (1985), who mentioned that clockwise strategy probably depends on motor-based stratagems. Although no gross, permanent motor deficits arise after bilateral HP lesions, an association between HP activity and motor acts has long been described (O' Keefe and Conway, 1978). In non-human primates, movement-related responses have been reported in anterior, but not middle or posterior, portions of the HP (Colombo, Fernandez et al. 1998). In rodents, ventral, but not dorsal HP stimulation increases locomotion by engaging the Nucleus Accumbens and mesolimbic dopamine system (Bardgett and Henry,1999). Therefore, if the sequential strategy is a motor-based

strategy, the major involvement of the ventral HP in regulating motor behaviour could explain the major involvement of the ventral HP in the use of the sequential strategy.

Functional dissociation between dorsal and ventral hippocampus in regulating anxiety-like behaviour.

The HP has not only a role in memory. Recent studies reported that HP lesions also result in reduced anxiety. A growing number of studies now suggest that these distinct functions may be associated with different HP subregions. Dorsal HP has a preferential role in spatial learning and memory while ventral HP has a preferential role in brain processes associated with anxiety-related behaviours. In a previous study, ventral HP lesions caused behavioural effects resembling those induced by benzodiazepines consistent with a reduction of anxiety (Mc Naughton and Gray, 2000). Other studies confirmed these results using behavioural paradigms measuring anxiety-like behaviors (Bannerman, Deacon et al. 2002). Rats with ventral HP excitotoxic lesions had a significantly higher percentage of visits to open arms than sham-operated rats and rats with dorsal lesions. Time spent on the open arms was also group-dependent (ventral lesions, 28.4%; dorsal lesions, 16.7%; sham surgery, 7.4%) (Kjelstrup, Tuvnes et al. 2002). In order to verify that our ventral HP lesion damaged the same region classically associated to emotional processes in literature, both dorsal HP and ventral HP lesion groups were tested on an elevated plus maze test. As already known from literature, our study confirmed that dorsal HP lesion had no effects on neural processes regulating anxiety while ventral HP lesion caused an important reduction of anxiety, which is evident by the increased time spent in the open arms in ventral HP lesion group. Different connectivity of the dorsal and ventral HP may account for this functional difference. Ventral HP is closely connected to the amygdala. Bidirectional connections have been documented between the ventral HP and the basolateral complex of the amygdala (Petrovich, Scicli et al. 2000). The amygdala plays a pivotal role in the control of fear expression. The ventral HP is also closely associated with subcortical structures which contribute to the hypothalamic–pituitary–adrenal axis. In fact, the crucial role of the ventral HP in defensive and fear-related behaviour is also confirmed by lower defecation and lower release of corticosterone in rats with lesions in the ventral HP when confined to a brightly lit environment (Kjelstrup, Tuvnes et al.2002).

Conclusions

This study shed novel light on the role of the dorsal and ventral HP in WMC paradigms. A specific involvement of the dorsal HP in the object working memory in condition of high memory load has been reported. The major connectivity of this part of the HP with the perirhinal cortex, generally associated to object recognition memory (Barker, Bird et al. 2007) could explain this preferential involvement. On the contrary, a complementary role was found for the two regions in spatial WMC when the spatial information must be processed using an allocentric strategy. Both regions are involved when the load of spatial locations to remember is high suggesting that in low memory load conditions a spearing of one of the subregions can compensate for the dysfunction of the other part. In contrast, in conditions of high memory load the whole HP is recruited in the task. Conversely a dissociation between these two regions was found in processing egocentric spatial information confirming previous results. *c-fos* activation was found in the ventral HP in animals trained to use an egocentric strategy in a starmaze task (Fouquet et al., 2013). The major connection of the ventral part of the HP with the Nucleus Accumbens could explain its major involvement in the use of the egocentric sequential strategy, a motor-based strategy.

Study 2. A dorsal hippocampus disease mouse model: α-Synuclein overexpression in dorsal hippocampus

Aim

α-Synuclein is a presynaptic protein of unknown function that is mutated in some familial cases of Parkinson's disease and it is the major component of Lewy bodies found in Parkinson's disease, dementia with Lewy bodies (Spillantini, Schmidt et al. 1997) and in the Lewy body variant of Alzheimer's disease (Forstl, Burns et al. 1993). α-Synuclein is a component of the Lewy neurites found in the HP of patients with dementia with Lewy bodies, a common late-life dementia that is clinically similar to Alzheimer's disease (Spillantini, Crowther et al. 1998). α-Synuclein expression was also found in the granular and polymorphic layers of the dentate gyrus and in the CA2 and CA3 fields at 30 and 43 weeks in a genetic mouse model of Parkinson's disease (A53T transgenic mice) (Flores-Cuadrado, Ubeda-Banon et al. 2016). In Parkinson's disease patients, a significantly higher density of α-Synuclein was observed in the CA2. Dementia in Parkinson's disease is associated to the onset of HP pathology in stage IV of the disease (Flores-Cuadrado, Ubeda-Banon et al. 2016). Emerging data in animal models (Costa, Sgobio et al. 2012) for non-motor symptoms in Parkinson's disease, such as cognitive impairment and behavioural disorders, support a role of the HP in this pathology. Cognitive functions such as visuospatial function, visual recognition memory, conditional associative learning, and verbal memory were not restored with dopamine replacement treatment in patients with Parkinson's disease suggesting the involvement of non-dopaminergic systems. HP dysfunction might explain the high prevalence of dementia in advanced stages of Parkinson's disease. A neuropathological study (Churchyard and Lees 1997) has shown an association between the severity of cognitive impairment in Parkinson's disease and the extent of deposition of Lewy bodies and Lewy neurites in the HP, which suggests a crucial role of HP structures in cognitive impairment in the disease. Most studies showed that HP atrophy in patients with Parkinson's disease with or without dementia was associated with cognitive impairment (Laakso, Partanen et al. 1996).

Transgenic mice expressing wild-type human α-Synuclein have been traditionally used as mouse model of Parkinson's disease (Masliah, Rockenstein et al. 2000). The use of adeno-associated virus (AAV) or lentivirus (LV) to create mouse models of Parkinson's disease is becoming an efficient tool overcoming the limits associated to transgenic α-Synuclein mouse models that failed to display clear dopaminergic cell loss and dopamine-dependent behavioral deficits. They were used to express α-

Synuclein in substantia nigra initially in rats (Kirik, Rosenblad et al. 2002), then in mice (Theodore, Cao et al. 2008). These vectors expressing human wildtype or mutated a-Synuclein in midbrain dopamine neurons reproduced the same features of the human pathology as cellular and axonal pathologies involving abnormal protein aggregation, neuronal dysfunction, and cell death. The neurodegeneration is time-dependent. Here we used the AAV-mediated approach to directly overexpress human a-Synuclein in dorsal HP of CD1 mice to create a mouse model of dorsal HP pathology and then evaluate the effects on object WMC. A contextual fear conditioning test was also performed to characterize this model.

Materials and methods

Recombinant AAV viral vector

The recombinant adeno-associated viral vector (rAAV) 2/6 expressing human (hu) α-Synuclein (rAAV2/6-hu-α-Syn) and the recombinant adeno-associated viral vector (rAAV) 2/6 expressing GFP (rAAV2/6-GFP) vectors were kindly provided by Professor Anders Björklund and were the same used in studies previously described (Decressac, Mattsson et al. 2012, Alvarsson, Caudal et al. 2016),. The expression of the transgene is driven by the synapsin-1 (Syn) promoter and it is enhanced using a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) in both vectors. Vector production was performed as described previously (Decressac M. et al., 2012). The injected vector titer was $7.7x10^{14}$ genome copies/ml (gc/ml) for both rAAV2/6-hu-α-syn and rAAV2/6-GFP vectors.

Subjects

Experiments were performed in outbred CD1 male mice 9-16 weeks old at the time of surgery (Charles River, Italy, RRID: rid_000091). Mice were housed in groups of 3–5 subjects, with *ad libitum* access to food and water and under a 12 h light/dark cycle. All procedures related to animal care and treatments were conformed to the guidelines and policies of the European Communities Council and were approved by the Italian Ministry of Health.

Surgery

Bilateral injections of rAAV2/6-hu-α-Syn and rAAV2/6-GFP were performed for a group tested 5 weeks post-injection (r-AAV-hu-α-Syn group, n = 11; r-AAV-GFP group, n = 6) and a group tested 22 weeks post-injection (r-AAV-hu-α-Syn group, n = 15; r-AAV-GFP group, n = 9). The two time points were chosen on the basis of a previous study in my laboratory where the rAAV 2/6-hu-α-Syn was injected in the substantia nigra of CD1 mice and the time-dependent progression of the pathology was evaluated. The surgical procedure was the same described in the first study. CD1 mice were placed in a stereotaxic frame (Kopf Instruments, USA) and vector solution was bilaterally injected within the dorsal HP using a 0.2 mm-gauge stainless steel injector connected via PE 20 tubing to a 5 μl Hamilton syringe. In all experimental groups, the rAAV was injected at a volume of 0.5 μl/side at a rate of 0.2 μl/min and the needle was left in place for an additional 10 min period before it was slowly retracted. The stereotaxic coordinates were the same used for the NMDA-mediated lesion of the dorsal HP.

Behavioral procedures

6 DOT/6 IOT

6 DOT and 6 IOT were performed as described in Sannino et al. (Sannino, Russo et al. 2012). The 6 DOT allows to measure WM in high memory load condition. In the 6-DOT mice were isolated for 15 minutes in a waiting cage before testing and then subjected to a habituation period of 10 minutes in an empty arena (35 x 47 x 60 cm), T1 phase. Habituation period allows assessing motor impairment. After 1 min spent in their waiting cage, they were subjected to the study phase (T2 phase), during which they were allowed to explore 6 different objects for 10 min or for a maximum of 120 sec of total objects exploration. Exploration was defined as the time in which the nose was in contact (< 2 cm from the object) with the object. After 1 min of intertrial interval (ITI), animals were subjected to the test phase, T3 phase during which the objects were replaced with identical copies of the familiar objects and a new object. Two different new objects were used between animals and the position of the new object was changed across animals in a random order to avoid any bias linked to the object used or to the position. Animals were allowed to explore the objects for 5 min. The animal's behaviour was recorded for 5 min by a video-tracking system (Any-maze, Stoelting, USA) and analyzed by a trained observer. In the 6 IOT animals were exposed to identical copies of the same objects. This allowed to increase the number of objects without increasing the memory load. The 6 IOT is the control task for the 6 DOT. In this case, T1 phase was identical to T1 phase in the 6 DOT. In the T2 phase, mice were allowed to explore objects for 5 min or for a maximum of 35 sec of total objects exploration. In the T3 phase, the objects were replaced with identical copies of the familiar objects and a new object. Animals were allowed to explore the objects for 5 min.

The same animals were exposed to 6 DOT and to 6 IOT 8 days apart.

Exploration of each object was measured. New object discrimination was considered to occur when the new object was explored significantly more than all the other familiar objects, tested with Duncan *post hoc* test.

Fear conditioning test

The conditioning chamber consisted of a modified shuttle box (Automatic Reflex Conditioner model 7531, Ugo Basile, Italy) made of gray opaque Plexiglas. One of the compartments (22×22×25 cm) of

the chamber was used for contextual fear conditioning. Mice were placed in the conditioning chamber for 374 seconds in the first day (training day) where they received a 2 seconds electric foot shock (0.5mA) at 188, 250 and 312 seconds from the beginning of the test, after which they were kept for an additional minute in the chamber before being returned to their home cages. In the second day (test day), they were placed in the chamber for 360 seconds without electric foot shock. Freezing, defined as a stereotyped crouching position with complete immobility of the animal, except for the movements necessary for breathing, scored during the subsequent re-exposure to the context (in the test day) was used as memory index.

Immunofluorescence

Primary antibody of human α-Synuclein (hu-α-Syn) (1:1000, sc-12767, Santa Cruz Biotechnology) in combination with anti-NeuN (1:500 MAB377, Millipore) was used. Brain sections were incubated overnight at 4°C with a PBS solution containing BSA 0.1% and the primary antibody (or a combination of two primary antibodies). After rinsing in PBS, the sections were incubated for 2 hours with the proper secondary antibody (or a mixture of them): goat anti-mouse Alexa 488 and goat anti-rabbit Alexa 568 (1:300 Millipore), respectively. The specificity of immunoreaction was controlled by omitting primary antibodies. No immunostaining was observed under these conditions. Images of dorsal HP were taken (4x magnification) using a fully motorized confocal microscope Nikon A1. Only animals with correct injection placements, verified by analysing immunofluorescence staining of consecutive coronal brain sections, were included in the statistical analysis. A counting for NeuN positive (NeuN +) neurons was performed on four matched brain slices per animal representative of the dorsal HP. Number of NeuN + cells was counted in a specific area (520 x 800 pixels) on the dorsal CA1 and in a specific area (930 x 930 pixels) on the dorsal CA2/CA3 using Image J (NIH, 1.47). The same areas were used to count cells in all animals.

Statistical analysis

Statistical analysis was performed using a one-way ANOVA with treatment as between factor (2 levels: r-AAV-hu-α-Syn and r-AAV-GFP) to analyse percentage of NeuN + neurons, total objects exploration during the T2 phase in both 6 DOT and 6 IOT, distance travelled during the T1 phase in both 6 DOT and 6 IOT and percentage of freezing time in fear conditioning test. A two-way ANOVA for repeated measures was applied, with the same between group factor (2 levels: r-AAV-hu-α-Syn

and r-AAV-GFP) and objects (6 levels: New, F1, F2, F3, F4, F5) as repeated measures to analyse total exploration during T3 phase in both 6 DOT and 6 IOT. Duncan *post-hoc* test was used when appropriate and the statistical significance was set at $p < 0.05$.

Results

α- Synuclein overexpression caused neuronal death 22 weeks post-injection

In order to verify the correct injection of the AAV overexpressing human α-Synuclein, an immunofluorescence for α-Synuclein was performed. α-Synuclein and GFP were found in dorsal CA1 HP of injected mice. Representative coronal brain sections from r-AAV-hu-α-Syn injected mice and r-AAV-GFP injected mice in both 5 and 22 weeks post-injection groups are shown in Fig. 35. Only animals with the correct injection of the vector evident as green signal in the HP were included in our analysis. r-AAV-GFP injected mice were our control animals.

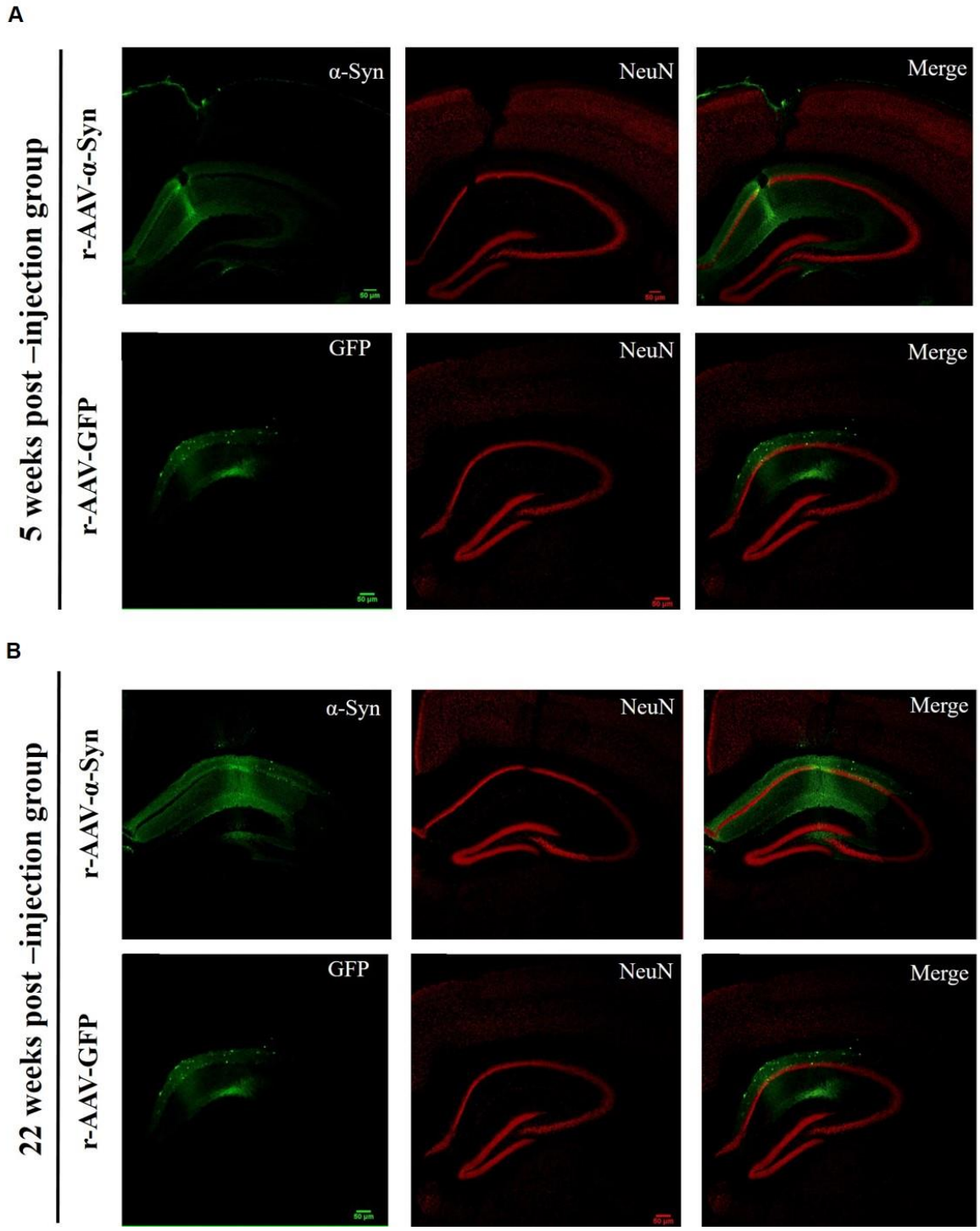


Fig. 35 α- Synuclein overexpression in dorsal HP. *Representative coronal sections from r-AAV-hu-α-Syn injected mice and r-AAV-GFP injected mice at 5 weeks (**A**) and 22 weeks (**B**) post-injections. Immunofluorescence against α-Synuclein (visualized with an antibody specific for human α- Syn, in green) shows cytoplasmatic expression of the protein in dorsal CA1 region (in the top). The nuclei are stained using an antibody against the neuronal nuclear protein NeuN (in red). The Sections in the second line in **A** and in **B** are taken from r-AAV-GFP injected mice.*

α- Synuclein overexpression did not cause neuronal death, measured as number of NeuN + cells, in both dorsal CA1 (one-way ANOVA, treatment $F_{(1,14)} = 2.218$; $p = 0.1586$) and dorsal CA3 (treatment $F_{(1,14)} = 1.457$; $p = 0.2473$) regions at 5 weeks post-injection. Neuronal death was found only in dorsal CA2/CA3 (treatment $F_{(1,13)} = 5.691$; $p = 0.0330$) but not in dorsal CA1 (treatment $F_{(1,13)} = 0.733 = 0.4075$) at 22 weeks post-injection (Fig.36).

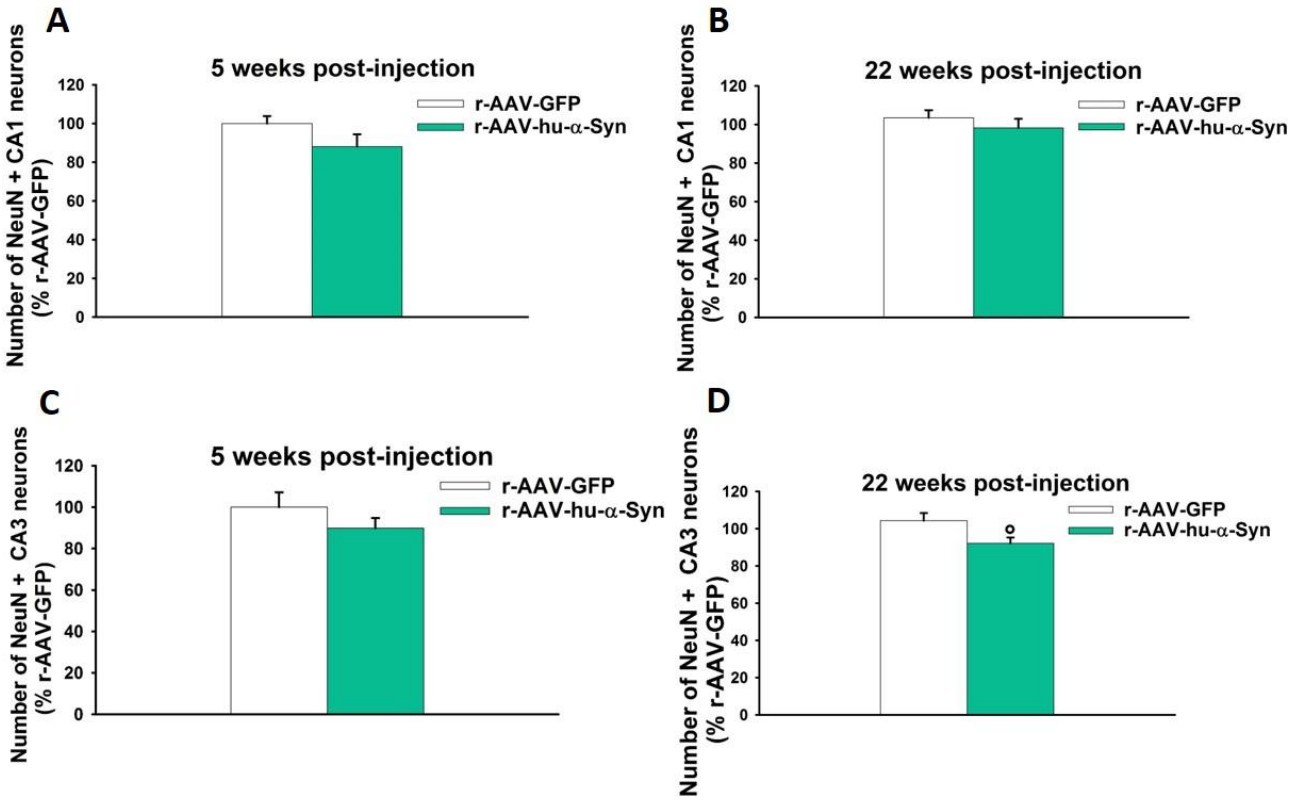


Fig. 36 Neuronal death in dorsal CA3 in r-AAV-hu-α-Syn injected mice at 22 weeks post-injection. *Percentage of NeuN + (NeuN positive) neurons in dorsal CA1 of r-AAV-hu-α-Syn injected mice relative to r-AAV-GFP injected mice at 5 weeks post-injection (**A**) and at 22 weeks post-injection (**B**). Percentage of NeuN + neurons in dorsal CA3 of r-AAV-hu-α-Syn injected mice relative to r-AAV-GFP injected mice at 5 weeks post-injection (C) and at 22 weeks post-injection (**C**). Data are expressed as mean ±SEM. ° p < 0.5 vs r-AAV-GFP mice. Duncan post-hoc analysis.*

α- Synuclein overexpression in the dorsal hippocampus impaired object WMC before the onset of neuronal loss.

r-AAV-hu-α-Syn and r-AAV-GFP injected mice were tested in 6 DOT/6IOT, 5 and 22 weeks after the virus infection to evaluate the performance in an early stage and in a late stage post-injection. First, we analysed the objects exploration during the T3 phase in the 6 DOT in the group tested 5 weeks after the injection. Duncan *post-hoc* analysis showed that only r-AAV-GFP injected mice discriminated the new object as compared to familiar ones while r-AAV-hu-α-Syn injected mice did

not discriminate the new object from the others. r-AAV-hu-α-Syn injected mice did not differ from the r-AAV-GFP injected mice for the total objects exploration time during the T2 phase (one-way ANOVA, treatment $F_{(1,15)} = 0.059$; $p = 0.8106$) and for the distance travelled in the arena during the T1 phase (one-way ANOVA, treatment $F_{(1,15)} = 0.165$; $p = 0.6905$) (Fig. 37 A-B-E). On the contrary, r-AAV-hu-α-Syn injected mice, as well as r-AAV GFP injected mice, performed normally in the 6 IOT as both groups explored the new object significantly more than the other familiar ones. No group difference was found in the total objects exploration during the T2 phase (one-way ANOVA, treatment $F_{(1,15)} = 0.006$; $p = 0.9396$) and in the distance during T1 phase in the 6 IOT (one-way ANOVA, treatment $F_{(1,15)} = 0.009$; $p = 0.9253$) (Fig. 37 C-D-F).

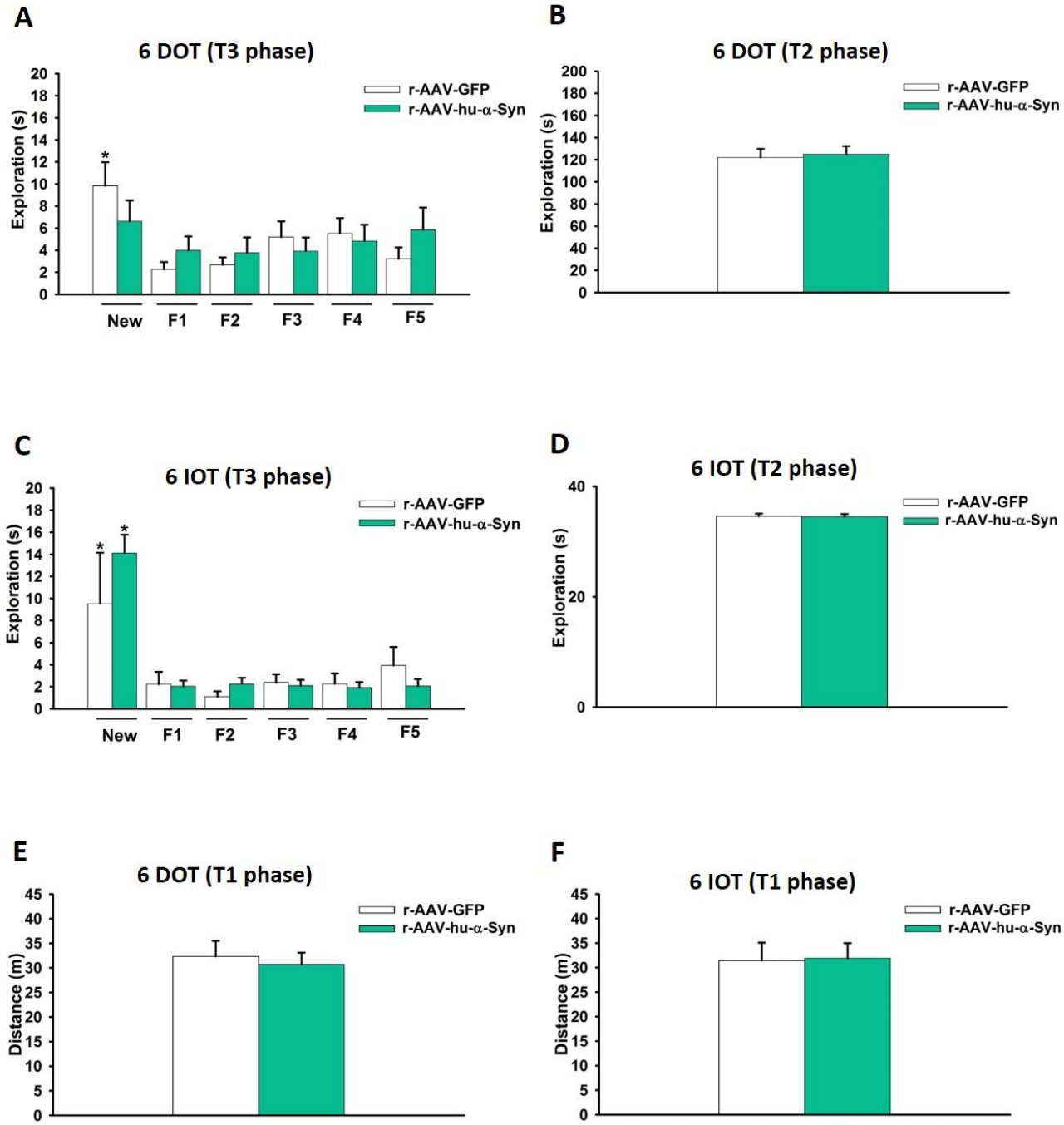


Fig. 37 r-AAV-hu-α-Syn injection in the dorsal HP impaired new object discrimination in the 6 DOT, already 5 weeks post-injection. *New and familiar (F1-F5) objects exploration during the test phase (T3 phase, **A**-**C**) and total objects exploration during the study phase (T2 phase, **B**-**D**) in r-AAV-GFP injected mice and in r-AAV-hu-α-Syn injected mice in the 6 DOT and in the 6 IOT. Distance travelled in the arena in the T1 phase of the 6 DOT (**E**) and 6 IOT (**F**). Data are expressed as mean ±SEM. * p <0.05 new vs. all the others familiar objects. Duncan post-hoc analysis.*

The same result was found in the experimental group tested 22 weeks after the injection. r-AAV-hu-α-Syn injected mice did not discriminate the new object from the familiar ones. Duncan *post-hoc* analysis revealed the new object discrimination only in the r-AAV-GFP group (Fig. 38 A). r-AAV-hu-α-Syn injection did not affect the objects exploration during the T2 phase (one-way ANOVA, treatment $F_{(1,22)} = 0.001$; $p = 0.9728$) and the distance travelled in the arena during the T1 phase (one-way ANOVA, treatment $F_{(1,15)} = 0.594$; $p = 0.4491$) (Fig. 38 B-E). No impairment was found in the 6 IOT in both the T3 phase and the T2 phase (one-way ANOVA, treatment $F_{(1,22)} = 0.629$; $p = 0.4360$) (Fig. 38 C-D). No difference was found in the distance during T1 phase (one-way ANOVA, treatment $F_{(1,22)} = 0.033$; $p = 0.8584$) (Fig.38 F).

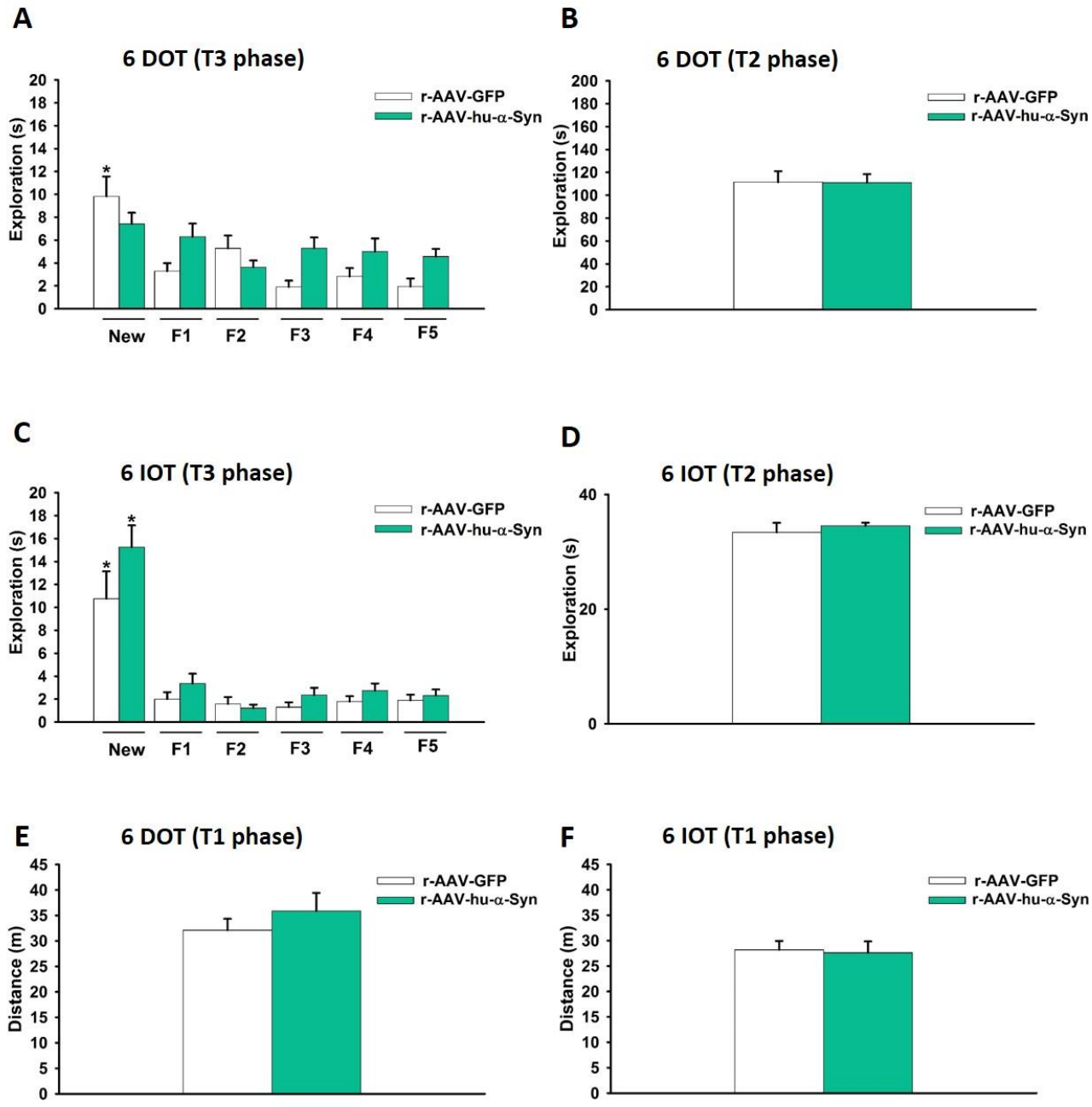


Fig. 38 Object WMC defect in r-AAV-hu-α-Syn injected mice at 22 weeks post-injection. *New and familiar (F) objects exploration during the test phase (T3 phase, **A-C**) and total objects exploration during the study phase (T2 phase, **B-D**) in r-AAV-GFP injected mice and in r-AAV-hu-α-Syn injected mice in the 6 DOT and in the 6 IOT. Distance travelled in the arena in the T1 phase in r-AAV-GFP injected mice and in r-AAV-hu-α-Syn injected mice in the 6 DOT (**E**) and in the 6 IOT (**F**). Data are expressed as mean ±SEM. * p <0.05 new vs. all of the others familiar objects. Duncan post-hoc analysis.*

These results show that the overexpression of the human wild-type α-Synuclein in the dorsal HP of normal mice lead to a time-dependent neuronal loss, which selectively affected the CA3 subregion of the HP. They show for the first time that α-Synuclein overexpression in the HP leads to impaired

object WMC before the onset of neuronal loss. The data suggest a high sensitivity of WMC task, which allow to detect a defect already at an early stage of the damage.

α- Synuclein overexpression in the dorsal hippocampus impaired contextual fear conditioning.

Fear conditioning is an experimental procedure in which an aversive unconditioned stimulus (US), such as footshock, is presented in association with a neutral conditioned stimulus (CS), often an auditory tone. Stimuli present in the environment where a US is presented but not explicitly paired with the US may also acquire aversive properties and thereby elicit conditioned emotional responses (Blanchard and Blanchard 1972). Such responses are said to be conditioned to static, background, or contextual cues. Recent studies reported that the amygdala is involved in both CS and contextual fear conditioning, but the HP is only involved in the contextual fear conditioning (Selden, Everit et al. 1991). HP lesions interfere with the acquisition of the contextual fear conditioning (Phillips and LeDoux 1992). The effect of the overexpression of α- Synuclein in the dorsal HP on the conditioning of freezing responses to contextual cues were analysed by performing a two-way ANOVA for repeated measures using the phases of the test (training and test) as repeated measure and the treatment factor as between factor. Overexpression of α- Synuclein in dorsal HP reduced the freezing response in the test phase at 5 and 22 weeks post-injection [(treatment $F_{(1,15)} = 5.836$; $p = 0.0289$; treatment x phases (training and test) $F_{(1,15)} = 6.136$; $p = 0.0256$, 5 weeks post-injection group), (treatment $F_{(1,20)} = 5.151$; $p = 0.0344$; treatment x phases $F_{(1,20)} = 8.499$; $p = 0.0086$, 22 weeks post-injection group)] (Fig. 39).

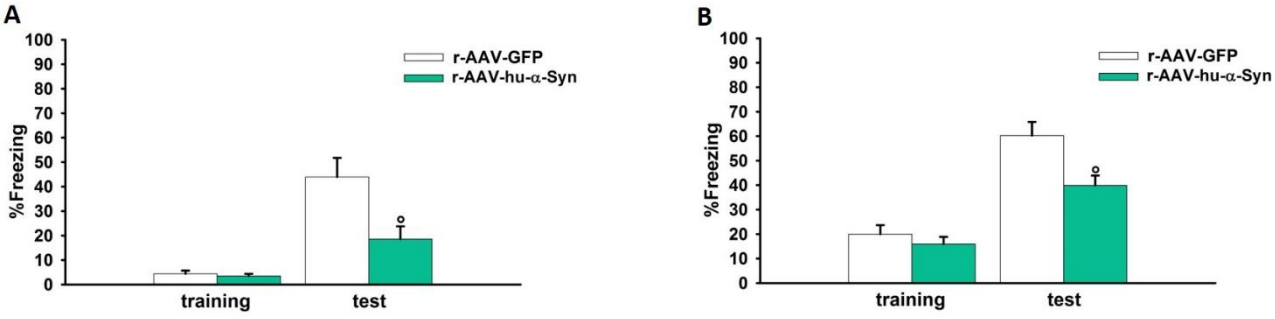


Fig. 39 Contextual fear conditioning defect in r-AAV-hu-α-Syn injected mice, 5 and 22 weeks post-injection. *Mean percentage of freezing time during the training phase and the test phase in the contextual fear conditioning at 5 weeks* **(A)** *and 22 weeks* **(B)** *post-injection. Data are expressed as mean ±SEM. ° $p < 0.05$ vs. r-AAV-GFP injected mice. Duncan post-hoc analysis.*

Discussion

Overexpression of human wild-type α-Synuclein in midbrain dopamine neurons through recombinant adeno-associated virus (AAV) has become in the last years an efficient tool to develop an authentic model of Parkinson's disease (PD). α-Synuclein is a key player in in the pathogenesis of familial PD (Polymeropoulos, Lavedan et al. 1997) and it is the major component of the characteristic protein inclusions, known as Lewy bodies and dystrophic neurites that develop over time in the brains of PD patients (Spillantini, Schmidt et al. 1997). α-Synuclein overexpression induces several neurophatological changes. Overexpression of human wild-type α-Synuclein is sufficient to trigger an inflammatory response that induce a-Synuclein aggegation, and cell death in nigral DA neurons (Gao, Kotzbauer et al. 2008). Neuronal damage is caused by the formation of toxic oligomers induced by oxidative damage. The accumulation of citoplasmatic a-Synuclein viral vector mediated interferes with the dopamine synthesis, storage and release and with the dopamine transporter (DAT) negatively regulating it activity with the following reduced dopamine uptake. a-Synuclein overexpression was found in the HP in A53T transgenic mice, a mouse model of familiar Parkinson's disease at 30 and 43 weeks. In Parkinson's disease patients, a significantly higher density of α-synuclein was observed in the CA2 arising around the onset of HP pathology responsible of dementia in Parkinson's disease (Flores-Cuadrado, Ubeda-Banon et al. 2016). Moreover, memory impairment in dementia with Lewy bodies correlated with a-Synuclein pathology in CA2 subfield of the HP (Adamowicz, Roy et al. 2017).

A particular advantage of AAV vector delivery is that it is possible to target a-Synuclein overexpression to selected brain structures, also outside the substantia nigra. For example, PD shows not only motor symptoms but also cognitive decline, mood disturbances, olfactory deficits, and balance and sleep disturbances that can be associate to others brain regions. On the basis of these evidence of an a-Synuclein pathology in HP in Parkinson's disease patients in a late stage of the disease and in Dementia with Lewy bodies patients, we created a dorsal HP disease mouse model through a specific injection of human α-Synuclein in this region and then we analysed the effects. We found an impairment in the 6 DOT that exactly reproduced the same defect caused by the dorsal HP lesion validating the role of this region in object WMC. This suggests that, WMC deficits documented in PD patients (Wilson, Kaszniak et al. 1980) could be now associated to this specific HP region. The defect in 6 DOT was already present at 5 weeks post-injection but neuronal death was observed only at 22 weeks post-injection in dorsal CA3 region. This could be due to neuronal alterations occuring before neuronal death. In fact, axonal pathology and the formation of α-Synuclein positive

cytoplasmatic inclusions have been found early after the viral vector injection. (Chung, Koprich et al. 2009). Overexpression of α-Synuclein in the dorsal HP caused an impairment in the contextual fear conditioning test confirming the results found in lesion studies. Numerous studies have shown that lesions of the dorsal HP disrupted the acquisition of conditioned freezing in response to contextual cues (Lee, Khoshaghideh et al. 2004). Therefore, overexpression of α-Synuclein in the dorsal HP induced the defect in objects WMC and in contextual fear conditioning already evident at 5 weeks post-injection, an early stage demonstrating that they are mediated by this HP region.

Conclusions

All together these results demonstrated that the overexpression of α-Synuclein in the dorsal HP is sufficient to induce an object WMC defect and a contextual fear conditioning defect already at 5 weeks post-injection before the neuronal death occurs (22 weeks post-injection). This demonstrates also that object WMC is so strictly dorsal HP-dependent to be sensible to an early phase of the damage. Previous findings obtained by overexpressing α-Synuclein in the mesencephalon showed a time-dependent death of dopaminergic neurons 22 weeks after the viral infection, as evidenced after HP injection. However, dopaminergic neuronal loss was associated to a worsening of the behavioural symptoms (Giordano, Iemolo et al. 2017). In this case, no worsening is observed for instance in the 6 DOT, thus suggesting that early cognitive deficits in this brain site-specific model of α-Synuclein accumulation are due to synaptic dysfunction rather than to neuronal loss.

In this way we created a mouse model of dorsal HP pathology that reproduces WMC deficits found in Parkinson's disease (Wilson, Kaszniak et al. 1980) identifying the dorsal HP as neuronal basis of this defect.

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